# DATA SHEET-V1
# REMBRANDT® HPV SCREENING
## DETECTION ASSAY

CE IVD

PanPath

**Ref**

| | | |
|---|---|---|
| **A100K.0101** | Σ | 10-100 T |
| **A100K.0105** | Σ | 10-100 T |
| **A100K.9901** | Σ | 10-100 T |
| **A100K.9905** | Σ | 10-100 T |

## Intended use

i) The REMBRANDT® HPV screening detection assay is an in-vitro diagnostics medical device intended for the qualitative detection of the human papillomavirus (HPV) type 6/11, 16/18, and 31/33, by means of chromogenic DNA *in situ* hybridization.

ii) The REMBRANDT® HPV screening detection assay is intended for the qualitative detection of the human papillomavirus (HPV) type 6/11, 16/18, and 31/33 in fixed cells and FFPE tissue sections. The clinical interpretation of the results should not be established on the basis of a single test result. A precise diagnosis, in fact, should take into consideration clinical history, symptoms, as well as other possible test data.

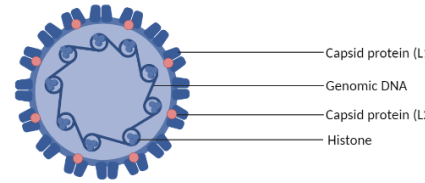
iii) The intended users are qualified laboratory employees in cytology and/or pathology. The product is intended for professional use.

## Clinical relevance

The most common sexual transmitted infection (STI) is infection with the human papillomavirus (HPV) (Elissa Meites, 2021). HPV is the main causative agent of cervical cancer in women, the fourth most common cancer in women worldwide. HPV is a small (~8 kb), dsDNA virus that infects the epithelium and mucosa epithelium (Choi & Park, 2016; Heise, 2003). Low-risk type infections like 6 or 11 could lead to benign or low-grade cell abnormalities like genital warts (National cancer institute, 2023). High-risk infections like 16, 18, 31, or are detected in 99% of cervical precancers. Type 16 and 18 account for approximately 66% of all cervical cancers. Infection with HPV is not likely to cause cervical cancer by itself, but is considered to be necessary for the development of cervical cancer. Cervical cancer is not the only cancer associated with high-risk HPV infection. Vulva, vagina, penis, and anus cancers are also related with high-risk HPV infections (Elissa Meites, 2021).

## Probe specification

The REMBRANDT® HPV screening detection assay is designed to target the human papillomavirus (HPV) type 6/11, 16/18, and 31/33 by means of chromogenic DNA *in situ* hybridization (CISH). The complementary DNA probes are labelled with digoxigenin or biotin.
The REMBRANDT® HPV screening probes are pre-mixed in a hybridization mixture (formamide, dextran sulphate and SSC) and are ready to use solutions.




## Test principle

In a chromogenic DNA in situ hybridization assay, a double stranded DNA oligonucleotide probe labelled with a hapten (digoxigenin or biotin) is used. The labelled dsDNA probe is diluted in a hybridization mixture. The hybridization mixture containing the dsDNA probe is added to the specimen. The probe DNA is able to hybridize to its complementary target sequences in the cells. The haptens need to be detected using conjugated antibodies. The HRP or AP conjugated antibodies are able to attach to the haptens. After incubation with the corresponding antibodies, a chromogenic-substrate reaction will allow visualization of HPV via bright field microscopy.

## Reagents provided

| Product name | Product number | Amount |
|---|---|---|
| Labelled probe (depending on label) | | |
| ●REMBRANDT® panHPV probes BIO or DIG detection | A100P.0100 or A100P.9900 | Σ 1 mL each |
| Conjugated antibodies (depending on probe and detection system) | | |
| ●REMBRANDT® aDIG-AP Fab conjugate | R003R.0000 | 15 mL |
| ●REMBRANDT® aDIG-HRP Fab conjugate | R004R.0000 | 15 mL |
| ●REMBRANDT® aBIO-AP Fab conjugate | R041R.0000 | 15 mL |
| ●REMBRANDT® ® aDIG-HRP Fab conjugate | R042R.0000 | 15 mL |
| Cchromogen substrate reaction mixture (depending on detection system) | | |
| ●REMBRANDT® AEC substrate and AEC buffer | R007R.0000<br>R010R.0000 | 2 mL<br>15 mL |

| | | |
|---|---|---|
| ●REMBRANDT® NBT/BCIP | R008R.0000 | 15 mL |
| Pepsin powder | R011R.0000 | 1 g |

Control probe positive/negative (depending on DIG or BIO assay)

| | | |
|---|---|---|
| ●REMBRANDT® DISH negative control DIG probe | Q001P.9900 | 1 mL |
| ●REMBRANDT® DISH positive control DIG probe | Q151P.9900 | 1 mL |
| ●REMBRANDT® DISH negative control BIO probe | Q001P.0100 | 1 mL |
| ●REMBRANDT® DISH positive control BIO probe | Q151P.0100 | 1 mL |

Counterstain (depending on detection system)

| | | |
|---|---|---|
| ●REMBRANDT® Methyl green counterstain | R016R.0000 | 15 mL |
| ●REMBRANDT® Nuclear fast red counterstain | R015R.0000 | 15 mL |

Provided for each detection system

| | | |
|---|---|---|
| REMBRANDT® Pepsin powder | R011R.0000 | 1 g |
| REMBRANDT® Pepsin diluent | R018R.0000 | 15 ml |
| REMBRANDT® PanWash (GC% <50%) | R013R.0000 | 15 mL |
| HPV 16 control slides | Q116C.0000 | 2 pcs |

## Assay procedure

REMBRANDT® HPV screening detection assay procedure for cytological specimen, frozen sections and FFPE tissue sections.

I. *Cytological specimen:*
Deposit the cells on coated glass slides and air dry for 30 minutes. Fix the slides with a cross-linking fixative (e.g. 4% para-formaldehyde) for 10 minutes at room temperature and rinse with PBS.
*FFPE tissue sections:*
For a detailed description of sample preparation and pre-treatment see: DISH Manual section 2.1.

II. Incubate both test and control slides (Q116C.0000) in pre-heated proteolytic work solution (prepare according to section 1.9 of the DISH Manual (R011R.000 + R018R.000)) at 37 ˚C. Paraffin-embedded sections (1.25 mg/ml) for 30 minutes or cytological specimen or frozen sections (100 µg/ml) for 10 minutes.

III. Flush wash slides in deionised water, followed by dehydration in graded ethanol series (ethanol 70%, 96%, 96%, 100%, 100%) 1 minute each and air-dry slides for 15 minutes.

**Do not treat more than 5 slides at the same time, because the temperature of the pre-heated solutions may drop dramatically, thus causing incomplete pre-treatment. Additionally, allow the slides to air-dry as recommended; otherwise sections will be lost.**

IV. Homogenize probe solution (AXXXP.YYY) and spin briefly. Apply 1 drop or 10-20 µl of probe solution to each specimen and the positive control specimen (Q116C.0000). Apply 1 drop or 20 µl of the negative control probe (Q001P.XXXX) to each negative control specimen and apply 1 drop or 20 µl of the positive control probe (Q151P.XXXX) to each positive control specimen.

V. Cover all specimens with a cover slip (avoid air bubbles).

VI. Place slides on a 80 ˚C hotplate and denature for 10 minutes.

**Do not denature more than 5 slides at the same time, because the temperature of the pre-heated solutions may drop dramatically, thus causing incomplete denaturation.**

VII. Transfer the slides into a moist environment and incubate for 16 hours at 37 ˚C.

VIII. Remove coverslips by soaking the slides in TBS buffer solution for 10 minutes at room temperature.

IX. Take the slides out, wipe off excess buffer and dry the edges using a lint-free cloth.

X. Apply 5-6 drops of PanWash >50% GC (R013R.0000) to each specimen and transfer the slides onto a 37 ˚C heating block. Incubate for 15 minutes. Rinse the slides 3 times for 1 minute in TBS buffer. Wipe off excess buffer and dry the edges using a lint-free cloth.

XI. Apply 2-3 drops of the appropriate conjugate (R0XXR.0000) to each specimen and transfer the slides onto a 37 ˚C heating block or slide warmer. Incubate for 30 minutes.

XII. Tap off excess detection reagent and rinse the slides in TBS buffer at room temperature.

XIII. Transfer the slides into a container with deionised water and soak for 1 minute.

XIV. Tap off excess water and dry around the edges using a lint-free cloth. Ensure that the specimen on the slide is not disrupted.

XV. Apply 2-3 drops of chromogen substrate reaction mixture to each specimen (prepare according to section 2.4 of DISH Manual). Transfer the slides onto a 37 ˚C heating block or slide warmer. Incubate in the dark for 5-15 minutes.

XVI. Tap off excess substrate solution and rinse slides 3 times for 1 minute in fresh deionised water. The slides are now ready to be mounted or counterstained.

**When a contrast colour is desired, the slides can be counterstained. If not, proceed to step XVI**

XVII. Wipe off excess reagent and apply 2-3 drops of Methyl green counterstain (R016R.0000) to each specimen. Incubate for at least 1 minute (longer incubation is possible and will yield in stronger staining).

XVIII. Tap off excess counterstain and rinse the slides briefly in deionised water.

XIX. Mount the slides by using an aqueous mounting medium. Interpret the results under a brightfield microscope.

## Interpretation of results

Hybridization of the REMBRANDT® HPV screening detection assay is viewed using a brightfield microscope. The detection of HPV is conducted by microscopic examination of cells. The stained cells that contain HPV DNA stand out bright against the de-stained cells. The REMBRANDT® HPV screening CISH procedure enables visual detection of HPV types within cells. First, check if the positive control shows colour precipitations the nucleus of the cells. In the test slides, start under low power magnification and focus on localisation and colour to see whether:

•The positivity (colour precipitation) is observed in conformity with nuclei or cytoplasm of tissue/cells of interest

•The colour has the right shade (no endogenous or formalin pigment)

Use high power magnification to see whether:
•The positive staining texture (granular, etc), demarcation and localisation are conform the positive control staining pattern

## Performance characteristics

**Analytical Sensitivity and Specificity**

The analytical sensitivity, specificity and precision were investigated within PanPaths analytical performance assessment. The results of the analytical performance study of the REMBRANDT® HPV screening detection assay are available upon request.

**Clinical performance**

The clinical sensitivity was determined for the REMBRANDT® HPV screening detection assay based on assessment of 50 samples with a known HPV status based on PCR. The sensitivity of the REMBRANDT® HPV screening detection assay was shown to be 85%. Clinical specificity was demonstrated by using samples (n=20) infected by similar viruses: HSV1, HSV2, VZV and CMV. The specificity of the REMBRANDT® HPV screening detection assay was shown to be 95%.

## Limitations of Procedure

i) The REMBRANDT® HPV screening detection assay is solely applicable for the detection of HPV DNA which may be present in cell preparations (cytological specimen i.e. interphase lymphocytes from peripheral blood samples or FFPE tissue sections).

ii) Either human tissue sections or human cytological preparations may be used. Samples must be fixed in buffered formalin or alcohol. In tissue sections are required, the sections should be prepared in a 4 µm thickness. Furthermore, the tissues should be glued to the glass slides with a bio-adhesive (e.g. organ silane), dried at room temperature, subsequently dried at 37 °C overnight and lastly completely deparaffinized in xylene and alcohol series and air dried.

iii) Cytological specimen should be prepared as required by the user, fixed with cytological fixation agent, rinsed in distilled water prior to the ISH procedure and air dried.

iv) Many factors can influence the performance of the ISH procedure. Failure in detection can be due to i.e. improper sampling, handling, the time lapse between tissue removal and fixation, the size of the tissue specimen in the fixation medium, the fixation time, processing fixed tissue, the thickness of the section, the bio-adhesive on the slide, deparaffinisation procedure, incubation times, proteolytic pre-treatment, detection reagents, incubation temperatures and interpretation of results.

v) The performance of the DISH procedure is also affected by the sensitivity of the method and the presence of HPV DNA. In case the limit of the sensitivity is reached a false negative reaction may be the result.

vi) The REMBRANDT® HPV screening detection assay results should not be relied on in case the sampling, sampling method, sample quality, sample preparation, reagents used, controls and procedure followed are not optimal or as described the working protocols.

vii) The clinical interpretation of the results should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history as well as data obtained from other molecular test (i.e. PCR).

viii) Therapeutic considerations based on the result of this test alone should not been taken. Results should be verified by other traditional diagnostic methods such as but not limited to clinical history, symptoms, as well as morphological data.

ix) The medical profession should be aware of risks and factors influencing the chromogenic signal intensity while interpretating the test result. Microscopy settings might influence the signal intensity and/or interpretation.

x) Laboratory personnel performing the test should be trained and knowledgeable to be able to interpret the test results.

## Storage and handling

Store kit and its contents at 2-8°C. Store the dissolved and aliquoted reagents at recommended temperatures. When used and stored as indicated, the kit is stable until the expiry date printed on the box.

| Product | Product number | Storage conditions |
|---|---|---|
| REMBRANDT® panHPV probe mix BIO or DIG detection | AXXXP.XXXX | 2-8 ˚C |
| REMBRANDT® Pepsin powder | R011R.0000 | Powder: 2-25˚C, ambient temperature<br>Dissolved: -20˚C |
| REMBRANDT® Pepsin diluent | R018R.0000 | Concentrated solution and diluted: 2-25˚C, ambient temperature |
| REMBRANDT® PanWash (GC% <50%) | R013R.0000 | 2-8 ˚C |
| REMBRANDT® NBT/BCIP substrate | R008R.0000 | 2-8 ˚C |
| REMBRANDT® AEC Substrate and AEC buffer | R007R.0000<br>R010R.0000 | 2-8 ˚C |
| REMBRANDT® Nuclear fast red counterstain | R015R.0000 | 2-8 ˚C |
| REMBRANDT® Methyl green counterstain | R016R.0000 | 2-8 ˚C |
| REMBRANDT® DISH negative or positive control probe | QXXXP.XXX | 2-8 ˚C |
| REMBRANDT® Positive control slides, paraffin embedded | QXXXC.0000 | 2-25 ˚C |

## Hazard statements

H315 - Causes skin irritation
H319 - Causes serious eye irritation
H351 - Suspected of causing cancer
H360D - May damage the unborn child
H373 - May cause damage to organs through prolonged or repeated exposure

## Precautionary Statements

P202 - Do not handle until all safety precautions have been read and understood
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P302 + P352 - IF ON SKIN: Wash with plenty of water and soap
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P308 + P313 - IF exposed or concerned: Get medical advice/attention
P362 + P364 - Take off contaminated clothing and wash it before reuse
P405 - Store locked up

## Additional information

**Product in combination with other devices**

The REMBRANDT® DNA probes are intended for stand-alone usage. The *in vitro* diagnostic is intended to be used in combination with standard cytological preparation methods, hot plate(s), stove(s), incubation device(s), water bath(s), temperature and incubation time control(s), proteolytic-, detection- and other reagents (not supplied with this reagent) and a microscope. The combination has been tested and validated. Since the standard cytological preparation methods, hot plate(s), stove(s), incubation device(s), water bath(s), temperature controls, incubation time control(s) and other not supplied reagents such as but not limited to proteolytic reagents, detection reagents and a microscope is not combined with the device as a product, conformity with the essential requirements is not applicable. Assay validation criteria are mentioned in *'Interpretation of the Results'* and are also depending on clinical state of the sample, which may influence the validation criteria.

For additional information regarding the REMBRANDT® assays, a manual is included which specifies the following subjects:

- Controls
- Materials required but not included
- Storage and shelf-life
- Performance precautions
- Preparations of reagents
- Specimen collection
- Quality control
- Trouble shooting guide

## Technical assistance

For technical assistance regarding the products performance, please contact info@panpath.nl or call +31 495499090. Visit our website for reprints of datasheets or additional documentation. www.panpath.nl

## Literature list

Abraham, R. T., & Weiss, A. (2004). Jurkat T cells and development of the T-cell receptor signalling

paradigm. *Nature Reviews Immunology*, *4*(4), 301–308. https://doi.org/10.1038/nri1330

Kuri, A., Jacobs, B. M., Jacobs, B. M., Vickaryous, N., Pakpoor, J., Middeldorp, J., Giovannoni, G., Dobson, R., & Dobson, R. (2020). Epidemiology of Epstein-Barr virus infection and infectious mononucleosis in the United Kingdom. *BMC Public Health*, *20*(1), 1–9. https://doi.org/10.1186/s12889-020-09049-x

Sheikh, T. I., & Qadri, I. (2011). Expression of EBV Encoded viral RNA 1, 2 and anti-inflammatory Cytokine (interleukin-10) in FFPE lymphoma specimens: A preliminary study for diagnostic implication in Pakistan. *Diagnostic Pathology*, *6*(1), 70. https://doi.org/10.1186/1746-1596-6-70

Yang, H. J., Huang, T. J., Yang, C. F., Peng, L. X., Liu, R. Y., Yang, G. Da, Chu, Q. Q., Huang, J. L., Liu, N., Huang, H. B., Zhu, Z. Y., Qian, C. N., & Huang, B. J. (2013). Comprehensive profiling of Epstein-Barr virus-encoded miRNA species associated with specific latency types in tumor cells. *Virology Journal*, *10*(1), 1. https://doi.org/10.1186/1743-422X-10-314

Yee, C., Krishnan-Hewlett, I., Baker, C. C., Schlegel, R., & Howley, P. M. (1985). Presence and expression of human papillomavirus sequences in human cervical carcinoma cell lines. *American Journal of Pathology*, *119*(3), 361–366.

Yoshizaki, T., Kondo, S., Wakisaka, N., Murono, S., Endo, K., Sugimoto, H., Nakanishi, S., Tsuji, A., & Ito, M. (2013). Pathogenic role of Epstein-Barr virus latent membrane protein-1 in the development of nasopharyngeal carcinoma. *Cancer Letters*, *337*(1), 1–7. https://doi.org/10.1016/j.canlet.2013.05.018

*Disclaimer: This document is valid until the product expiry on the kit label*

# DATA SHEET-V1
# REMBRANDT® HPV TYPING DETECTION ASSAY

CE IVD

PanPath

**Ref**

| | | |
|---|---|---|
| **A103K.0101** | Σ | 10-100 T |
| **A103K.0105** | Σ | 10-100 T |
| **A103K.9901** | Σ | 10-100 T |
| **A103K.9905** | Σ | 10-100 T |

## Intended use

i) The REMBRANDT® HPV typing detection assay is an in-vitro diagnostics medical device intended for the qualitative detection of the human papillomavirus (HPV) type 6/11, 16/18, or 31/33, by means of chromogenic DNA *in situ* hybridization.

ii) The REMBRANDT® HPV typing detection assay is intended for the qualitative detection of the human papillomavirus (HPV) type 6/11, 16/18, or 31/33 in fixed cells and FFPE tissue sections. The clinical interpretation of the results should not be established on the basis of a single test result. A precise diagnosis, in fact, should take into consideration clinical history, symptoms, as well as other possible test data.

iii) The intended users are qualified laboratory employees in cytology and/or pathology. The product is intended for professional use.

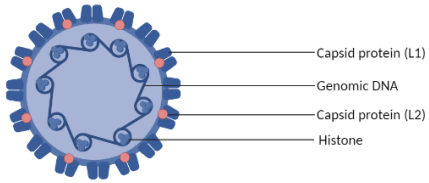
## Clinical relevance

The most common sexual transmitted infection (STI) is infection with the human papillomavirus (HPV) (Elissa Meites, 2021). HPV is the main causative agent of cervical cancer in women, the fourth most common cancer in women worldwide. HPV is a small (~8 kb), dsDNA virus that infects the epithelium and mucosa epithelium (Choi & Park, 2016; Heise, 2003). Low-risk type infections like 6 or 11 could lead to benign or low-grade cell abnormalities like genital warts (National cancer institute, 2023). High-risk infections like 16, 18, 31, or are detected in 99% of cervical precancers. Type 16 and 18 account for approximately 66% of all cervical cancers. Infection with HPV is not likely to cause cervical cancer by itself, but is considered to be necessary for the development of cervical cancer. Cervical cancer is not the only cancer associated with high-risk HPV infection. Vulva, vagina, penis, and anus cancers are also related with high-risk HPV infections (Elissa Meites, 2021).

## Probe specification

The REMBRANDT® HPV typing detection assay is designed to target the human papillomavirus (HPV) type 6/11, 16/18, or 31/33 by means of chromogenic DNA *in situ* hybridization (CISH). The complementary DNA probes are labelled with digoxigenin or biotin.

The REMBRANDT® HPV typing probes are pre-mixed in a hybridization mixture (formamide, dextran sulphate and SSC) and are ready to use solutions.




## Test principle

In a chromogenic DNA in situ hybridization assay, a double stranded DNA oligonucleotide probe labelled with a hapten (digoxigenin or biotin) is used. The labelled dsDNA probe is diluted in a hybridization mixture. The hybridization mixture containing the dsDNA probe is added to the specimen. The probe DNA is able to hybridize to its complementary target sequences in the cells. The haptens need to be detected using conjugated antibodies. The HRP or AP conjugated antibodies are able to attach to the haptens. After incubation with the corresponding antibodies, a chromogenic-substrate reaction will allow visualization of HPV via bright field microscopy.

## Reagents provided

| Product name | Product number | Amount |
|---|---|---|
| Labelled probe (depending on label) | | |
| ● REMBRANDT® HPV type specific BIO DNA probe | A191P.0100<br>A192P.0100<br>A193P.0100 | Σ 1 mL each |
| ● REMBRANDT® HPV type specific DIG DNA probe | A191P.9900<br>A192P.9900<br>A193P.9900 | Σ 1 mL each |
| Conjugated antibodies (depending on probe and detection system) | | |
| ●REMBRANDT® aDIG-AP Fab conjugate | R003R.0000 | 15 mL |
| ●REMBRANDT® aDIG-HRP Fab conjugate | R004R.0000 | 15 mL |
| ●REMBRANDT® aBIO-AP Fab conjugate | R041R.0000 | 15 mL |
| ●REMBRANDT® ® aDIG-HRP Fab conjugate | R042R.0000 | 15 mL |

| | | |
|---|---|---|
| Cchromogen substrate reaction mixture (depending on detection system) | | |
| ●REMBRANDT® AEC substrate and AEC buffer | R007R.0000 | 2 mL |
| | R010R.0000 | 15 mL |
| ●REMBRANDT® NBT/BCIP | R008R.0000 | 15 mL |
| Pepsin powder | R011R.0000 | 1 g |
| Control probe positive/negative (depending on DIG or BIO assay) | | |
| ●REMBRANDT® DISH negative control DIG probe | Q001P.9900 | 1 mL |
| ●REMBRANDT® DISH positive control DIG probe | Q151P.9900 | 1 mL |
| ●REMBRANDT® DISH negative control BIO probe | Q001P.0100 | 1 mL |
| ●REMBRANDT® DISH positive control BIO probe | Q151P.0100 | 1 mL |
| Counterstain (depending on detection system) | | |
| ●REMBRANDT® Methyl green counterstain | R016R.0000 | 15 mL |
| ●REMBRANDT® Nuclear fast red counterstain | R015R.0000 | 15 mL |
| Provided for each detection system | | |
| REMBRANDT® Pepsin powder | R011R.0000 | 1 g |
| REMBRANDT® Pepsin diluent | R018R.0000 | 15 ml |
| REMBRANDT® PanWash (GC% <50%) | R013R.0000 | 15 mL |
| HPV 16 control slides | Q116C.0000 | 2 pcs |

## Assay procedure

REMBRANDT® HPV typing detection assay procedure for cytological specimen, frozen sections and FFPE tissue sections.

I. *Cytological specimen:*
Deposit the cells on coated glass slides and air dry for 30 minutes. Fix the slides with a cross-linking fixative (e.g. 4% para-formaldehyde) for 10 minutes at room temperature and rinse with PBS.
*FFPE tissue sections:*
For a detailed description of sample preparation and pre-treatment see: DISH Manual section 2.1.

II. Incubate both test and control slides (Q116C.0000) in pre-heated proteolytic work solution (prepare according to section 1.9 of the DISH Manual (R011R.000 + R018R.000)) at 37 ˚C. Paraffin-embedded sections (1.25 mg/ml) for 30 minutes or cytological specimen or frozen sections (100 µg/ml) for 10 minutes.

III. Flush wash slides in deionised water, followed by dehydration in graded ethanol series (ethanol 70%, 96%, 96%, 100%, 100%) 1 minute each and air-dry slides for 15 minutes.

**Do not treat more than 5 slides at the same time, because the temperature of the pre-heated solutions may drop dramatically, thus causing incomplete pre-treatment. Additionally, allow the slides to air-dry as recommended; otherwise sections will be lost.**

IV. Homogenize probe solution (AXXXP.YYY) and spin briefly. Apply 1 drop or 10-20 µl of probe solution to each specimen and the positive control specimen (Q116C.0000). Apply 1 drop or 20 µl of the negative control probe (Q001P.XXXX) to each negative control specimen and apply 1 drop or 20 µl of the positive control probe (Q151P.XXXX) to each positive control specimen.

V. Cover all specimens with a cover slip (avoid air bubbles).

VI. Place slides on a 80 ˚C hotplate and denature for 10 minutes.

**Do not denature more than 5 slides at the same time, because the temperature of the pre-heated solutions may drop dramatically, thus causing incomplete denaturation.**

VII. Transfer the slides into a moist environment and incubate for 16 hours at 37 ˚C.

VIII. Remove coverslips by soaking the slides in TBS buffer solution for 10 minutes at room temperature.

IX. Take the slides out, wipe off excess buffer and dry the edges using a lint-free cloth.

X. Apply 5-6 drops of PanWash >50% GC (R013R.0000) to each specimen and transfer the slides onto a 37 ˚C heating block. Incubate for 15 minutes. Rinse the slides 3 times for 1 minute in TBS buffer. Wipe off excess buffer and dry the edges using a lint-free cloth.

XI. Apply 2-3 drops of the appropriate conjugate (R0XXR.0000) to each specimen and transfer the slides onto a 37 ˚C heating block or slide warmer. Incubate for 30 minutes.

XII. Tap off excess detection reagent and rinse the slides in TBS buffer at room temperature.

XIII. Transfer the slides into a container with deionised water and soak for 1 minute.

XIV. Tap off excess water and dry around the edges using a lint-free cloth. Ensure that the specimen on the slide is not disrupted.

XV. Apply 2-3 drops of chromogen substrate reaction mixture to each specimen (prepare

according to section 2.4 of DISH Manual). Transfer the slides onto a 37 ˚C heating block or slide warmer. Incubate in the dark for 5-15 minutes.

XVI. Tap off excess substrate solution and rinse slides 3 times for 1 minute in fresh deionised water. The slides are now ready to be mounted or counterstained.

**When a contrast colour is desired, the slides can be counterstained. If not, proceed to step XVI**

XVII. Wipe off excess reagent and apply 2-3 drops of Methyl green counterstain (R016R.0000) to each specimen. Incubate for at least 1 minute (longer incubation is possible and will yield in stronger staining).

XVIII. Tap off excess counterstain and rinse the slides briefly in deionised water.

XIX. Mount the slides by using an aqueous mounting medium. Interpret the results under a brightfield microscope.

## Interpretation of results

Hybridization of the REMBRANDT® HPV typing detection assay is viewed using a brightfield microscope. The detection of HPV is conducted by microscopic examination of cells. The stained cells that contain HPV DNA stand out bright against the de-stained cells. The REMBRANDT® HPV typing CISH procedure enables visual detection of different HPV types within cells. First, check if the positive control shows colour precipitations the nucleus of the cells. In the test slides, start under low power magnification and focus on localisation and colour to see whether:

•The positivity (colour precipitation) is observed in conformity with nuclei or cytoplasm of tissue/cells of interest

•The colour has the right shade (no endogenous or formalin pigment)

Use high power magnification to see whether:
•The positive staining texture (granular, etc), demarcation and localisation are conform the positive control staining pattern

## Performance characteristics

**Analytical Sensitivity and Specificity**

The analytical sensitivity, specificity and precision were investigated within PanPaths analytical performance assessment. The results of the analytical performance study of the REMBRANDT® HPV typing detection assay are available upon request.

**Clinical performance**

The clinical sensitivity was determined for the REMBRANDT® HPV typing detection assay based on assessment of 50 samples with a known HPV status based on PCR. The sensitivity of the REMBRANDT® HPV typing detection assay was shown to be 85%. Clinical specificity was demonstrated by using samples (n=20) infected by similar viruses: HSV1, HSV2, VZV and CMV. The specificity of the REMBRANDT® HPV typing detection assay was shown to be 95%.

## Limitations of Procedure

i) The REMBRANDT® HPV typing detection assay is solely applicable for the detection of HPV DNA which may be present in cell preparations (cytological specimen i.e. interphase lymphocytes from peripheral blood samples or FFPE tissue sections).

ii) Either human tissue sections or human cytological preparations may be used. Samples must be fixed in buffered formalin or alcohol. In tissue sections are required, the sections should be prepared in a 4 µm thickness. Furthermore, the tissues should be glued to the glass slides with a bio-adhesive (e.g. organ silane), dried at room temperature, subsequently dried at 37 °C overnight and lastly completely deparaffinized in xylene and alcohol series and air dried.

iii) Cytological specimen should be prepared as required by the user, fixed with cytological fixation agent, rinsed in distilled water prior to the ISH procedure and air dried.

iv) Many factors can influence the performance of the ISH procedure. Failure in detection can be due to i.e. improper sampling, handling, the time lapse between tissue removal and fixation, the size of the tissue specimen in the fixation medium, the fixation time, processing fixed tissue, the thickness of the section, the bio-adhesive on the slide, deparaffinisation procedure, incubation times, proteolytic pre-treatment, detection reagents, incubation temperatures and interpretation of results.

v) The performance of the DISH procedure is also affected by the sensitivity of the method and the presence of HPV DNA. In case the limit of the sensitivity is reached a false negative reaction may be the result.

vi) The REMBRANDT® HPV typing detection assay results should not be relied on in case the sampling, sampling method, sample quality, sample preparation, reagents used, controls and procedure followed are not optimal or as described the working protocols.

vii) The clinical interpretation of the results should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history as well as data obtained from other molecular test (i.e. PCR).

viii) Therapeutic considerations based on the result of this test alone should not been taken. Results should be verified by other traditional diagnostic methods such as but not limited to clinical history, symptoms, as well as morphological data.

ix) The medical profession should be aware of risks and factors influencing the chromogenic signal intensity

while interpretating the test result. Microscopy settings might influence the signal intensity and/or interpretation.
x) Laboratory personnel performing the test should be trained and knowledgeable to be able to interpret the test results.

## Storage and handling

Store kit and its contents at 2-8°C. Store the dissolved and aliquoted reagents at recommended temperatures. When used and stored as indicated, the kit is stable until the expiry date printed on the box.

| Product | Product number | Storage conditions |
|---|---|---|
| REMBRANDT® type specific HPV probe mix BIO or DIG detection | AXXXP.XXXX | 2-8 ˚C |
| REMBRANDT® Pepsin powder | R011R.0000 | Powder: 2-25˚C, ambient temperature<br><br>Dissolved: -20˚C |
| REMBRANDT® Pepsin diluent | R018R.0000 | Concentrated solution and diluted: 2-25˚C, ambient temperature |
| REMBRANDT® PanWash (GC% <50%) | R013R.0000 | 2-8 ˚C |
| REMBRANDT® NBT/BCIP substrate | R008R.0000 | 2-8 ˚C |
| REMBRANDT® AEC Substrate and AEC buffer | R007R.0000<br>R010R.0000 | 2-8 ˚C |
| REMBRANDT® Nuclear fast red counterstain | R015R.0000 | 2-8 ˚C |
| REMBRANDT® Methyl green counterstain | R016R.0000 | 2-8 ˚C |
| REMBRANDT® DISH negative or positive control probe | QXXXP.XXX | 2-8 ˚C |
| REMBRANDT® Positive control slides, paraffin embedded | QXXXC.0000 | 2-25 ˚C |

## Hazard statements

H315 - Causes skin irritation
H319 - Causes serious eye irritation
H351 - Suspected of causing cancer
H360D - May damage the unborn child
H373 - May cause damage to organs through prolonged or repeated exposure

## Precautionary Statements

P202 - Do not handle until all safety precautions have been read and understood
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P302 + P352 - IF ON SKIN: Wash with plenty of water and soap
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P308 + P313 - IF exposed or concerned: Get medical advice/attention
P362 + P364 - Take off contaminated clothing and wash it before reuse
P405 - Store locked up

## Additional information

**Product in combination with other devices**

The REMBRANDT® DNA probes are intended for stand-alone usage. The in vitro diagnostic is intended to be used in combination with standard cytological preparation methods, hot plate(s), stove(s), incubation device(s), water bath(s), temperature and incubation time control(s), proteolytic-, detection- and other reagents (not supplied with this reagent) and a microscope. The combination has been tested and validated. Since the standard cytological preparation methods, hot plate(s), stove(s), incubation device(s), water bath(s), temperature controls, incubation time control(s) and other not supplied reagents such as but not limited to proteolytic reagents, detection reagents and a microscope is not combined with the device as a product, conformity with the essential requirements is not applicable. Assay validation criteria are mentioned in *'Interpretation of the Results'* and are also depending on clinical state of the sample, which may influence the validation criteria.

For additional information regarding the REMBRANDT® assays, a manual is included which specifies the following subjects:

- Controls
- Materials required but not included
- Storage and shelf-life
- Performance precautions
- Preparations of reagents
- Specimen collection
- Quality control
- Trouble shooting guide

## Technical assistance

For technical assistance regarding the products performance, please contact info@panpath.nl or call +31 495499090. Visit our website for reprints of datasheets or additional documentation. www.panpath.nl

## Literature list


Abraham, R. T., & Weiss, A. (2004). Jurkat T cells and development of the T-cell receptor signalling paradigm. *Nature Reviews Immunology*, *4*(4), 301–308. https://doi.org/10.1038/nri1330

Kuri, A., Jacobs, B. M., Jacobs, B. M., Vickaryous, N., Pakpoor, J., Middeldorp, J., Giovannoni, G., Dobson, R., & Dobson, R. (2020). Epidemiology of Epstein-Barr virus infection and infectious mononucleosis in the United Kingdom. *BMC Public Health*, *20*(1), 1–9. https://doi.org/10.1186/s12889-020-09049-x

Sheikh, T. I., & Qadri, I. (2011). Expression of EBV Encoded viral RNA 1, 2 and anti-inflammatory Cytokine (interleukin-10) in FFPE lymphoma specimens: A preliminary study for diagnostic implication in Pakistan. *Diagnostic Pathology*, *6*(1), 70. https://doi.org/10.1186/1746-1596-6-70

Yang, H. J., Huang, T. J., Yang, C. F., Peng, L. X., Liu, R. Y., Yang, G. Da, Chu, Q. Q., Huang, J. L., Liu, N., Huang, H. B., Zhu, Z. Y., Qian, C. N., & Huang, B. J. (2013). Comprehensive profiling of Epstein-Barr virus-encoded miRNA species associated with specific latency types in tumor cells. *Virology Journal*, *10*(1), 1. https://doi.org/10.1186/1743-422X-10-314

Yee, C., Krishnan-Hewlett, I., Baker, C. C., Schlegel, R., & Howley, P. M. (1985). Presence and expression of human papillomavirus sequences in human cervical carcinoma cell lines. *American Journal of Pathology*, *119*(3), 361–366.

Yoshizaki, T., Kondo, S., Wakisaka, N., Murono, S., Endo, K., Sugimoto, H., Nakanishi, S., Tsuji, A., & Ito, M. (2013). Pathogenic role of Epstein-Barr virus latent membrane protein-1 in the development of nasopharyngeal carcinoma. *Cancer Letters*, *337*(1), 1–7. https://doi.org/10.1016/j.canlet.2013.05.018


*Disclaimer: This document is valid until the product expiry on the kit label*

# DATA SHEET-V1
# REMBRANDT® HPV SCREENING
## DETECTION ASSAY

RUO

PanPath

**Ref**

| | | |
|---|---|---|
| **A100K.0101** | Σ | 10-100 T |
| **A100K.0105** | Σ | 10-100 T |
| **A100K.9901** | Σ | 10-100 T |
| **A100K.9905** | Σ | 10-100 T |

## Intended use

i) The REMBRANDT® HPV screening detection assay is intended for the qualitative detection of the human papillomavirus (HPV) type 6/11, 16/18, and 31/33, by means of chromogenic DNA *in situ* hybridization.

ii) The REMBRANDT® HPV screening detection assay is intended for the qualitative detection of the human papillomavirus (HPV) type 6/11, 16/18, and 31/33 in fixed cells and FFPE tissue sections. The clinical interpretation of the results should not be established on the basis of this tests result.

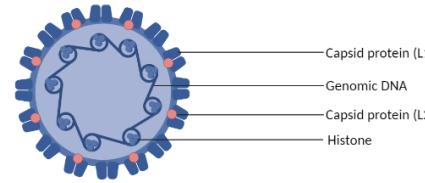
iii) The intended users are qualified laboratory employees in cytology and/or pathology. The product is intended for professional use.

## Clinical relevance

The most common sexual transmitted infection (STI) is infection with the human papillomavirus (HPV) (Elissa Meites, 2021). HPV is the main causative agent of cervical cancer in women, the fourth most common cancer in women worldwide. HPV is a small (~8 kb), dsDNA virus that infects the epithelium and mucosa epithelium (Choi & Park, 2016; Heise, 2003). Low-risk type infections like 6 or 11 could lead to benign or low-grade cell abnormalities like genital warts (National cancer institute, 2023). High-risk infections like 16, 18, 31, or are detected in 99% of cervical precancers. Type 16 and 18 account for approximately 66% of all cervical cancers. Infection with HPV is not likely to cause cervical cancer by itself, but is considered to be necessary for the development of cervical cancer. Cervical cancer is not the only cancer associated with high-risk HPV infection. Vulva, vagina, penis, and anus cancers are also related with high-risk HPV infections (Elissa Meites, 2021).

## Probe specification

The REMBRANDT® HPV screening detection assay is designed to target the human papillomavirus (HPV) type 6/11, 16/18, and 31/33 by means of chromogenic DNA *in situ* hybridization (CISH). The complementary DNA probes are labelled with digoxigenin or biotin.
The REMBRANDT® HPV screening probes are pre-mixed in a hybridization mixture (formamide, dextran sulphate and SSC) and are ready to use solutions.




## Test principle

In a chromogenic DNA in situ hybridization assay, a double stranded DNA oligonucleotide probe labelled with a hapten (digoxigenin or biotin) is used. The labelled dsDNA probe is diluted in a hybridization mixture. The hybridization mixture containing the dsDNA probe is added to the specimen. The probe DNA is able to hybridize to its complementary target sequences in the cells. The haptens need to be detected using conjugated antibodies. The HRP or AP conjugated antibodies are able to attach to the haptens. After incubation with the corresponding antibodies, a chromogenic-substrate reaction will allow visualization of HPV via bright field microscopy.

## Reagents provided

| Product name | Product number | Amount |
|---|---|---|
| Labelled probe (depending on label) | | |
| ●REMBRANDT® panHPV probes BIO or DIG detection | A100P.0100 or A100P.9900 | Σ 1 mL each |
| Conjugated antibodies (depending on probe and detection system) | | |
| ●REMBRANDT® aDIG-AP Fab conjugate | R003R.0000 | 15 mL |
| ●REMBRANDT® aDIG-HRP Fab conjugate | R004R.0000 | 15 mL |
| ●REMBRANDT® aBIO-AP Fab conjugate | R041R.0000 | 15 mL |
| ●REMBRANDT® ® aDIG-HRP Fab conjugate | R042R.0000 | 15 mL |
| Cchromogen substrate reaction mixture (depending on detection system) | | |
| ●REMBRANDT® AEC substrate and AEC buffer | R007R.0000<br>R010R.0000 | 2 mL<br>15 mL |

| | | |
|---|---|---|
| ●REMBRANDT® NBT/BCIP | R008R.0000 | 15 mL |
| Pepsin powder | R011R.0000 | 1 g |

Control probe positive/negative (depending on DIG or BIO assay)

| | | |
|---|---|---|
| ●REMBRANDT® DISH negative control DIG probe | Q001P.9900 | 1 mL |
| ●REMBRANDT® DISH positive control DIG probe | Q151P.9900 | 1 mL |
| ●REMBRANDT® DISH negative control BIO probe | Q001P.0100 | 1 mL |
| ●REMBRANDT® DISH positive control BIO probe | Q151P.0100 | 1 mL |

Counterstain (depending on detection system)

| | | |
|---|---|---|
| ●REMBRANDT® Methyl green counterstain | R016R.0000 | 15 mL |
| ●REMBRANDT® Nuclear fast red counterstain | R015R.0000 | 15 mL |

Provided for each detection system

| | | |
|---|---|---|
| REMBRANDT® Pepsin powder | R011R.0000 | 1 g |
| REMBRANDT® Pepsin diluent | R018R.0000 | 15 ml |
| REMBRANDT® PanWash (GC% <50%) | R013R.0000 | 15 mL |
| HPV 16 control slides | Q116C.0000 | 2 pcs |

## Assay procedure

REMBRANDT® HPV screening detection assay procedure for cytological specimen, frozen sections and FFPE tissue sections.

I. *Cytological specimen:*
Deposit the cells on coated glass slides and air dry for 30 minutes. Fix the slides with a cross-linking fixative (e.g. 4% para-formaldehyde) for 10 minutes at room temperature and rinse with PBS.
*FFPE tissue sections:*
For a detailed description of sample preparation and pre-treatment see: DISH Manual section 2.1.

II. Incubate both test and control slides (Q116C.0000) in pre-heated proteolytic work solution (prepare according to section 1.9 of the DISH Manual (R011R.000 + R018R.000)) at 37 ˚C. Paraffin-embedded sections (1.25 mg/ml) for 30 minutes or cytological specimen or frozen sections (100 µg/ml) for 10 minutes.

III. Flush wash slides in deionised water, followed by dehydration in graded ethanol series (ethanol 70%, 96%, 96%, 100%, 100%) 1 minute each and air-dry slides for 15 minutes.

**Do not treat more than 5 slides at the same time, because the temperature of the pre-heated solutions may drop dramatically, thus causing incomplete pre-treatment. Additionally, allow the slides to air-dry as recommended; otherwise sections will be lost.**

IV. Homogenize probe solution (AXXXP.YYY) and spin briefly. Apply 1 drop or 10-20 µl of probe solution to each specimen and the positive control specimen (Q116C.0000). Apply 1 drop or 20 µl of the negative control probe (Q001P.XXXX) to each negative control specimen and apply 1 drop or 20 µl of the positive control probe (Q151P.XXXX) to each positive control specimen.

V. Cover all specimens with a cover slip (avoid air bubbles).

VI. Place slides on a 80 ˚C hotplate and denature for 10 minutes.

**Do not denature more than 5 slides at the same time, because the temperature of the pre-heated solutions may drop dramatically, thus causing incomplete denaturation.**

VII. Transfer the slides into a moist environment and incubate for 16 hours at 37 ˚C.

VIII. Remove coverslips by soaking the slides in TBS buffer solution for 10 minutes at room temperature.

IX. Take the slides out, wipe off excess buffer and dry the edges using a lint-free cloth.

X. Apply 5-6 drops of PanWash >50% GC (R013R.0000) to each specimen and transfer the slides onto a 37 ˚C heating block. Incubate for 15 minutes. Rinse the slides 3 times for 1 minute in TBS buffer. Wipe off excess buffer and dry the edges using a lint-free cloth.

XI. Apply 2-3 drops of the appropriate conjugate (R0XXR.0000) to each specimen and transfer the slides onto a 37 ˚C heating block or slide warmer. Incubate for 30 minutes.

XII. Tap off excess detection reagent and rinse the slides in TBS buffer at room temperature.

XIII. Transfer the slides into a container with deionised water and soak for 1 minute.

XIV. Tap off excess water and dry around the edges using a lint-free cloth. Ensure that the specimen on the slide is not disrupted.

XV. Apply 2-3 drops of chromogen substrate reaction mixture to each specimen (prepare according to section 2.4 of DISH Manual). Transfer the slides onto a 37 ˚C heating block or slide warmer. Incubate in the dark for 5-15 minutes.

XVI. Tap off excess substrate solution and rinse slides 3 times for 1 minute in fresh deionised water. The slides are now ready to be mounted or counterstained.

**When a contrast colour is desired, the slides can be counterstained. If not, proceed to step XVI**

XVII. Wipe off excess reagent and apply 2-3 drops of Methyl green counterstain (R016R.0000) to each specimen. Incubate for at least 1 minute (longer incubation is possible and will yield in stronger staining).

XVIII. Tap off excess counterstain and rinse the slides briefly in deionised water.

XIX. Mount the slides by using an aqueous mounting medium. Interpret the results under a brightfield microscope.

## Interpretation of results

Hybridization of the REMBRANDT® HPV screening detection assay is viewed using a brightfield microscope. The detection of HPV is conducted by microscopic examination of cells. The stained cells that contain HPV DNA stand out bright against the de-stained cells. The REMBRANDT® HPV screening CISH procedure enables visual detection of HPV types within cells. First, check if the positive control shows colour precipitations the nucleus of the cells. In the test slides, start under low power magnification and focus on localisation and colour to see whether:

•The positivity (colour precipitation) is observed in conformity with nuclei or cytoplasm of tissue/cells of interest

•The colour has the right shade (no endogenous or formalin pigment)

Use high power magnification to see whether:
•The positive staining texture (granular, etc), demarcation and localisation are conform the positive control staining pattern

## Limitations of Procedure

i) The REMBRANDT® HPV screening detection assay is solely applicable for the detection of HPV DNA which may be present in cell preparations (cytological specimen i.e. interphase lymphocytes from peripheral blood samples or FFPE tissue sections).

ii) Either human tissue sections or human cytological preparations may be used. Samples must be fixed in buffered formalin or alcohol. In tissue sections are required, the sections should be prepared in a 4 µm thickness. Furthermore, the tissues should be glued to the glass slides with a bio-adhesive (e.g. organ silane), dried at room temperature, subsequently dried at 37 °C overnight and lastly completely deparaffinized in xylene and alcohol series and air dried.

iii) Cytological specimen should be prepared as required by the user, fixed with cytological fixation agent, rinsed in distilled water prior to the ISH procedure and air dried.

iv) Many factors can influence the performance of the ISH procedure. Failure in detection can be due to i.e. improper sampling, handling, the time lapse between tissue removal and fixation, the size of the tissue specimen in the fixation medium, the fixation time, processing fixed tissue, the thickness of the section, the bio-adhesive on the slide, deparaffinisation procedure, incubation times, proteolytic pre-treatment, detection reagents, incubation temperatures and interpretation of results.

v) The performance of the DISH procedure is also affected by the sensitivity of the method and the presence of HPV DNA. In case the limit of the sensitivity is reached a false negative reaction may be the result.

vi) The REMBRANDT® HPV screening detection assay results should not be relied on in case the sampling, sampling method, sample quality, sample preparation, reagents used, controls and procedure followed are not optimal or as described the working protocols.

vii) The clinical interpretation of the results should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history as well as data obtained from other molecular test (i.e. PCR).

viii) Therapeutic considerations based on the result of this test alone should not been taken. Results should be verified by other traditional diagnostic methods such as but not limited to clinical history, symptoms, as well as morphological data.

ix) The medical profession should be aware of risks and factors influencing the chromogenic signal intensity while interpretating the test result. Microscopy settings might influence the signal intensity and/or interpretation.

x) Laboratory personnel performing the test should be trained and knowledgeable to be able to interpret the test results.

## Storage and handling

Store kit and its contents at 2-8°C. Store the dissolved and aliquoted reagents at recommended temperatures. When used and stored as indicated, the kit is stable until the expiry date printed on the box.

| Product | Product number | Storage conditions |
|---|---|---|
| REMBRANDT® panHPV probe mix BIO or DIG detection | AXXXP.XXXX | 2-8 °C |
| REMBRANDT® Pepsin powder | R011R.0000 | Powder: 2-25°C, ambient temperature<br><br>Dissolved: -20°C |

| | | |
|---|---|---|
| REMBRANDT® Pepsin diluent | R018R.0000 | Concentrated solution and diluted: 2-25˚C, ambient temperature |
| REMBRANDT® PanWash (GC% <50%) | R013R.0000 | 2-8 ˚C |
| REMBRANDT® NBT/BCIP substrate | R008R.0000 | 2-8 ˚C |
| REMBRANDT® AEC Substrate and AEC buffer | R007R.0000 R010R.0000 | 2-8 ˚C |
| REMBRANDT® Nuclear fast red counterstain | R015R.0000 | 2-8 ˚C |
| REMBRANDT® Methyl green counterstain | R016R.0000 | 2-8 ˚C |
| REMBRANDT® DISH negative or positive control probe | QXXXP.XXX | 2-8 ˚C |
| REMBRANDT® Positive control slides, paraffin embedded | QXXXC.0000 | 2-25 ˚C |

## Hazard statements

H315 - Causes skin irritation
H319 - Causes serious eye irritation
H351 - Suspected of causing cancer
H360D - May damage the unborn child
H373 - May cause damage to organs through prolonged or repeated exposure

## Precautionary Statements

P202 - Do not handle until all safety precautions have been read and understood
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P302 + P352 - IF ON SKIN: Wash with plenty of water and soap
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P308 + P313 - IF exposed or concerned: Get medical advice/attention
P362 + P364 - Take off contaminated clothing and wash it before reuse
P405 - Store locked up

## Additional information

**Product in combination with other devices**

The REMBRANDT® DNA probes are intended for stand-alone usage. The assay is intended to be used in combination with standard cytological preparation methods, hot plate(s), stove(s), incubation device(s), water bath(s), temperature and incubation time control(s), proteolytic-, detection- and other reagents (not supplied with this reagent) and a microscope. The combination has been tested and validated. Since the standard cytological preparation methods, hot plate(s), stove(s), incubation device(s), water bath(s), temperature controls, incubation time control(s) and other not supplied reagents such as but not limited to proteolytic reagents, detection reagents and a microscope is not combined with the device as a product, conformity with the essential requirements is not applicable. Assay validation criteria are mentioned in *'Interpretation of the Results'* and are also depending on clinical state of the sample, which may influence the validation criteria.

For additional information regarding the REMBRANDT® assays, a manual is included which specifies the following subjects:

- Controls
- Materials required but not included
- Storage and shelf-life
- Performance precautions
- Preparations of reagents
- Specimen collection
- Quality control
- Trouble shooting guide

## Technical assistance

For technical assistance regarding the products performance, please contact info@panpath.nl or call +31 495499090. Visit our website for reprints of datasheets or additional documentation. www.panpath.nl

## Literature list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J. (2013). Comprehensive profiling of Epstein-Barr virus-encoded miRNA species associated with specific latency types in tumor cells. *Virology Journal*, *10*(1), 1. https://doi.org/10.1186/1743-422X-10-314

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*Disclaimer: This document is valid until the product expiry on the kit label*

# DATA SHEET-V1
# REMBRANDT® HPV TYPING
## DETECTION ASSAY

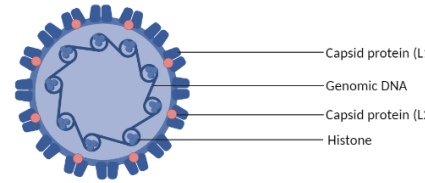
RUO

Pan Path

**Ref**

| | | |
|---|---|---|
| **A103K.0101** | Σ | 10-100 T |
| **A103K.0105** | Σ | 10-100 T |
| **A103K.9901** | Σ | 10-100 T |
| **A103K.9905** | Σ | 10-100 T |

The REMBRANDT® HPV typing probes are pre-mixed in a hybridization mixture (formamide, dextran sulphate and SSC) and are ready to use solutions.




## Intended use

i) The REMBRANDT® HPV typing detection assay is intended for the qualitative detection of the human papillomavirus (HPV) type 6/11, 16/18, or 31/33, by means of chromogenic DNA *in situ* hybridization.

ii) The REMBRANDT® HPV typing detection assay is intended for the qualitative detection of the human papillomavirus (HPV) type 6/11, 16/18, or 31/33 in fixed cells and FFPE tissue sections. The clinical interpretation of the results should not be established on the basis of this test result.

iii) The intended users are qualified laboratory employees in cytology and/or pathology. The product is intended for professional use.

## Clinical relevance

The most common sexual transmitted infection (STI) is infection with the human papillomavirus (HPV) (Elissa Meites, 2021). HPV is the main causative agent of cervical cancer in women, the fourth most common cancer in women worldwide. HPV is a small (~8 kb), dsDNA virus that infects the epithelium and mucosa epithelium (Choi & Park, 2016; Heise, 2003). Low-risk type infections like 6 or 11 could lead to benign or low-grade cell abnormalities like genital warts (National cancer institute, 2023). High-risk infections like 16, 18, 31, or are detected in 99% of cervical precancers. Type 16 and 18 account for approximately 66% of all cervical cancers. Infection with HPV is not likely to cause cervical cancer by itself, but is considered to be necessary for the development of cervical cancer. Cervical cancer is not the only cancer associated with high-risk HPV infection. Vulva, vagina, penis, and anus cancers are also related with high-risk HPV infections (Elissa Meites, 2021).

## Probe specification

The REMBRANDT® HPV typing detection assay is designed to target the human papillomavirus (HPV) type 6/11, 16/18, or 31/33 by means of chromogenic DNA *in situ* hybridization (CISH). The complementary DNA probes are labelled with digoxigenin or biotin.

## Test principle

In a chromogenic DNA in situ hybridization assay, a double stranded DNA oligonucleotide probe labelled with a hapten (digoxigenin or biotin) is used. The labelled dsDNA probe is diluted in a hybridization mixture. The hybridization mixture containing the dsDNA probe is added to the specimen. The probe DNA is able to hybridize to its complementary target sequences in the cells. The haptens need to be detected using conjugated antibodies. The HRP or AP conjugated antibodies are able to attach to the haptens. After incubation with the corresponding antibodies, a chromogenic-substrate reaction will allow visualization of HPV via bright field microscopy.

## Reagents provided

| Product name | Product number | Amount |
|---|---|---|
| Labelled probe (depending on label) | | |
| ● REMBRANDT® HPV type specific BIO DNA probe | A191P.0100<br>A192P.0100<br>A193P.0100 | Σ 1 mL each |
| ● REMBRANDT® HPV type specific DIG DNA probe | A191P.9900<br>A192P.9900<br>A193P.9900 | Σ 1 mL each |
| Conjugated antibodies (depending on probe and detection system) | | |
| ●REMBRANDT® aDIG-AP Fab conjugate | R003R.0000 | 15 mL |
| ●REMBRANDT® aDIG-HRP Fab conjugate | R004R.0000 | 15 mL |
| ●REMBRANDT® aBIO-AP Fab conjugate | R041R.0000 | 15 mL |
| ●REMBRANDT® ® aDIG-HRP Fab conjugate | R042R.0000 | 15 mL |

Cchromogen substrate reaction mixture (depending on detection system)

| | | |
|---|---|---|
| ●REMBRANDT® AEC substrate and AEC buffer | R007R.0000<br>R010R.0000 | 2 mL<br>15 mL |
| ●REMBRANDT® NBT/BCIP | R008R.0000 | 15 mL |
| Pepsin powder | R011R.0000 | 1 g |
| Control probe positive/negative (depending on DIG or BIO assay) | | |
| ●REMBRANDT® DISH negative control DIG probe | Q001P.9900 | 1 mL |
| ●REMBRANDT® DISH positive control DIG probe | Q151P.9900 | 1 mL |
| ●REMBRANDT® DISH negative control BIO probe | Q001P.0100 | 1 mL |
| ●REMBRANDT® DISH positive control BIO probe | Q151P.0100 | 1 mL |
| Counterstain (depending on detection system) | | |
| ●REMBRANDT® Methyl green counterstain | R016R.0000 | 15 mL |
| ●REMBRANDT® Nuclear fast red counterstain | R015R.0000 | 15 mL |
| Provided for each detection system | | |
| REMBRANDT® Pepsin powder | R011R.0000 | 1 g |
| REMBRANDT® Pepsin diluent | R018R.0000 | 15 ml |
| REMBRANDT® PanWash (GC% <50%) | R013R.0000 | 15 mL |
| HPV 16 control slides | Q116C.0000 | 2 pcs |

## Assay procedure

REMBRANDT® HPV typing detection assay procedure for cytological specimen, frozen sections and FFPE tissue sections.

I. *Cytological specimen:*
Deposit the cells on coated glass slides and air dry for 30 minutes. Fix the slides with a cross-linking fixative (e.g. 4% para-formaldehyde) for 10 minutes at room temperature and rinse with PBS.
*FFPE tissue sections:*
For a detailed description of sample preparation and pre-treatment see: DISH Manual section 2.1.

II. Incubate both test and control slides (Q116C.0000) in pre-heated proteolytic work solution (prepare according to section 1.9 of the DISH Manual (R011R.000 + R018R.000)) at 37 ˚C. Paraffin-embedded sections (1.25 mg/ml) for 30 minutes or cytological specimen or frozen sections (100 µg/ml) for 10 minutes.

III. Flush wash slides in deionised water, followed by dehydration in graded ethanol series (ethanol 70%, 96%, 96%, 100%, 100%) 1 minute each and air-dry slides for 15 minutes.

**Do not treat more than 5 slides at the same time, because the temperature of the pre-heated solutions may drop dramatically, thus causing incomplete pre-treatment. Additionally, allow the slides to air-dry as recommended; otherwise sections will be lost.**

IV. Homogenize probe solution (AXXXP.YYY) and spin briefly. Apply 1 drop or 10-20 µl of probe solution to each specimen and the positive control specimen (Q116C.0000). Apply 1 drop or 20 µl of the negative control probe (Q001P.XXXX) to each negative control specimen and apply 1 drop or 20 µl of the positive control probe (Q151P.XXXX) to each positive control specimen.

V. Cover all specimens with a cover slip (avoid air bubbles).

VI. Place slides on a 80 ˚C hotplate and denature for 10 minutes.

**Do not denature more than 5 slides at the same time, because the temperature of the pre-heated solutions may drop dramatically, thus causing incomplete denaturation.**

VII. Transfer the slides into a moist environment and incubate for 16 hours at 37 ˚C.

VIII. Remove coverslips by soaking the slides in TBS buffer solution for 10 minutes at room temperature.

IX. Take the slides out, wipe off excess buffer and dry the edges using a lint-free cloth.

X. Apply 5-6 drops of PanWash >50% GC (R013R.0000) to each specimen and transfer the slides onto a 37 ˚C heating block. Incubate for 15 minutes. Rinse the slides 3 times for 1 minute in TBS buffer. Wipe off excess buffer and dry the edges using a lint-free cloth.

XI. Apply 2-3 drops of the appropriate conjugate (R0XXR.0000) to each specimen and transfer the slides onto a 37 ˚C heating block or slide warmer. Incubate for 30 minutes.

XII. Tap off excess detection reagent and rinse the slides in TBS buffer at room temperature.

XIII. Transfer the slides into a container with deionised water and soak for 1 minute.

XIV. Tap off excess water and dry around the edges using a lint-free cloth. Ensure that the specimen on the slide is not disrupted.

XV. Apply 2-3 drops of chromogen substrate reaction mixture to each specimen (prepare according to section 2.4 of DISH Manual). Transfer the slides onto a 37 ˚C heating block or slide warmer. Incubate in the dark for 5-15 minutes.

XVI. Tap off excess substrate solution and rinse slides 3 times for 1 minute in fresh deionised water. The slides are now ready to be mounted or counterstained.

**When a contrast colour is desired, the slides can be counterstained. If not, proceed to step XVI**

XVII. Wipe off excess reagent and apply 2-3 drops of Methyl green counterstain (R016R.0000) to each specimen. Incubate for at least 1 minute (longer incubation is possible and will yield in stronger staining).

XVIII. Tap off excess counterstain and rinse the slides briefly in deionised water.

XIX. Mount the slides by using an aqueous mounting medium. Interpret the results under a brightfield microscope.

## Interpretation of results

Hybridization of the REMBRANDT® HPV typing detection assay is viewed using a brightfield microscope. The detection of HPV is conducted by microscopic examination of cells. The stained cells that contain HPV DNA stand out bright against the de-stained cells. The REMBRANDT® HPV typing CISH procedure enables visual detection of different HPV types within cells. First, check if the positive control shows colour precipitations the nucleus of the cells. In the test slides, start under low power magnification and focus on localisation and colour to see whether:

•The positivity (colour precipitation) is observed in conformity with nuclei or cytoplasm of tissue/cells of interest

•The colour has the right shade (no endogenous or formalin pigment)

Use high power magnification to see whether:
•The positive staining texture (granular, etc), demarcation and localisation are conform the positive control staining pattern

## Limitations of Procedure

i) The REMBRANDT® HPV typing detection assay is solely applicable for the detection of HPV DNA which may be present in cell preparations (cytological specimen i.e. interphase lymphocytes from peripheral blood samples or FFPE tissue sections).

ii) Either human tissue sections or human cytological preparations may be used. Samples must be fixed in buffered formalin or alcohol. In tissue sections are required, the sections should be prepared in a 4 µm thickness. Furthermore, the tissues should be glued to the glass slides with a bio-adhesive (e.g. organ silane), dried at room temperature, subsequently dried at 37 °C overnight and lastly completely deparaffinized in xylene and alcohol series and air dried.

iii) Cytological specimen should be prepared as required by the user, fixed with cytological fixation agent, rinsed in distilled water prior to the ISH procedure and air dried.

iv) Many factors can influence the performance of the ISH procedure. Failure in detection can be due to i.e. improper sampling, handling, the time lapse between tissue removal and fixation, the size of the tissue specimen in the fixation medium, the fixation time, processing fixed tissue, the thickness of the section, the bio-adhesive on the slide, deparaffinisation procedure, incubation times, proteolytic pre-treatment, detection reagents, incubation temperatures and interpretation of results.

v) The performance of the DISH procedure is also affected by the sensitivity of the method and the presence of HPV DNA. In case the limit of the sensitivity is reached a false negative reaction may be the result.

vi) The REMBRANDT® HPV typing detection assay results should not be relied on in case the sampling, sampling method, sample quality, sample preparation, reagents used, controls and procedure followed are not optimal or as described the working protocols.

vii) The clinical interpretation of the results should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history as well as data obtained from other molecular test (i.e. PCR).

viii) Therapeutic considerations based on the result of this test alone should not been taken. Results should be verified by other traditional diagnostic methods such as but not limited to clinical history, symptoms, as well as morphological data.

ix) The medical profession should be aware of risks and factors influencing the chromogenic signal intensity while interpretating the test result. Microscopy settings might influence the signal intensity and/or interpretation.

x) Laboratory personnel performing the test should be trained and knowledgeable to be able to interpret the test results.

## Storage and handling

Store kit and its contents at 2-8°C. Store the dissolved and aliquoted reagents at recommended temperatures. When used and stored as indicated, the kit is stable until the expiry date printed on the box.

| Product | Product number | Storage conditions |
|---|---|---|
| REMBRANDT® type specific HPV probe mix BIO or DIG detection | AXXXP.XXXX | 2-8 ˚C |
| REMBRANDT® Pepsin powder | R011R.0000 | Powder: 2-25˚C, ambient temperature<br><br>Dissolved: -20˚C |

| | | |
|---|---|---|
| REMBRANDT® Pepsin diluent | R018R.0000 | Concentrated solution and diluted: 2-25˚C, ambient temperature |
| REMBRANDT® PanWash (GC% <50%) | R013R.0000 | 2-8 ˚C |
| REMBRANDT® NBT/BCIP substrate | R008R.0000 | 2-8 ˚C |
| REMBRANDT® AEC Substrate and AEC buffer | R007R.0000 R010R.0000 | 2-8 ˚C |
| REMBRANDT® Nuclear fast red counterstain | R015R.0000 | 2-8 ˚C |
| REMBRANDT® Methyl green counterstain | R016R.0000 | 2-8 ˚C |
| REMBRANDT® DISH negative or positive control probe | QXXXP.XXX | 2-8 ˚C |
| REMBRANDT® Positive control slides, paraffin embedded | QXXXC.0000 | 2-25 ˚C |

## Hazard statements

H315 - Causes skin irritation
H319 - Causes serious eye irritation
H351 - Suspected of causing cancer
H360D - May damage the unborn child
H373 - May cause damage to organs through prolonged or repeated exposure

## Precautionary Statements

P202 - Do not handle until all safety precautions have been read and understood
P280 - Wear protective gloves/protective clothing/eye protection/face protection
P302 + P352 - IF ON SKIN: Wash with plenty of water and soap
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P308 + P313 - IF exposed or concerned: Get medical advice/attention
P362 + P364 - Take off contaminated clothing and wash it before reuse
P405 - Store locked up

## Additional information

**Product in combination with other devices**

The REMBRANDT® DNA probes are intended for stand-alone usage. The assay is intended to be used in combination with standard cytological preparation methods, hot plate(s), stove(s), incubation device(s), water bath(s), temperature and incubation time control(s), proteolytic-, detection- and other reagents (not supplied with this reagent) and a microscope. The combination has been tested and validated. Since the standard cytological preparation methods, hot plate(s), stove(s), incubation device(s), water bath(s), temperature controls, incubation time control(s) and other not supplied reagents such as but not limited to proteolytic reagents, detection reagents and a microscope is not combined with the device as a product, conformity with the essential requirements is not applicable. Assay validation criteria are mentioned in *'Interpretation of the Results'* and are also depending on clinical state of the sample, which may influence the validation criteria.

For additional information regarding the REMBRANDT® assays, a manual is included which specifies the following subjects:

- Controls
- Materials required but not included
- Storage and shelf-life
- Performance precautions
- Preparations of reagents
- Specimen collection
- Quality control
- Trouble shooting guide

## Technical assistance

For technical assistance regarding the products performance, please contact info@panpath.nl or call +31 495499090. Visit our website for reprints of datasheets or additional documentation. www.panpath.nl

## Literature list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*Disclaimer: This document is valid until the product expiry on the kit label*