

REMBRANDT®*In situ* Hybridisation and Detection***RISH & AP Detection Kit-v5*****Immaterial property information**

The probes in this product are labelled with the Universal Linkage System (ULS™). The Universal Linkage System (ULS™) technology is covered by an international patent family for the linkage of any label to bio-organic molecules, owned by KREATECH Biotechnology BV, The Netherlands. This product or the use of this product may be covered by one or more patents of KREATECH Biotechnology BV, including, but not restricted to, the following: EP 0539466; US 5,580,990; US 5,714,327; WO 92/01699; WO 96/35696; WO 98/15564.

Digoxigenin (DIG) labeling and detection is protected by international patents of Roche Molecular Biochemicals. This product is sold under a license of Roche Molecular Biochemicals. This product or the use of this product may be covered by one or more patents of Roche Molecular Biochemicals, including the following: EP 0324474, US 5,354,657.

REMBRANDT® is a registered trade name of PanPath BV, Amsterdam, The Netherlands.

Purchase does not include the right to exploit this product commercially and any commercial use without the explicit authorization of PanPath BV is prohibited.

<i>RISH-AP kit for the detection of</i>	Biotin Label product code	Digoxigenin label product code	# Assays
Epstein-Barr Encoded Small RNAs (EBER)	A500K.0105	A500K.9905	40
Kappa/Lambda light Chain RNAs	C600K.0105	C600K.9905	40

PanPath

Science Park Amsterdam
Kruislaan 406
1098 SM Amsterdam
The Netherlands
Tel. +31 20 751 2030
Fax +31 20 751 2031
E-mail info@panpath.nl
Web site www.panpath.nl

Contents

	<u>page</u>
Chapter 1 Introduction	03
1.1 Intended use	03
1.2 The ISH principle	03
1.3 Controls	03
1.4 Contents of the REMBRANDT® kit	03
1.5 Materials required but not included	04
1.6 Storage and shelf life	04
1.7 Safety precautions	04
1.8 Performance precautions	05
1.9 Preparations of reagents in advance	05
1.10 Preparation of the proteolytic work solution	05
Chapter 2 REMBRANDT® RISH & AP Detection protocol	06
2.1 Specimen collection and pre-treatment	06
2.2 Proteolytic treatment	06
2.3 Hybridisation procedure	07
2.4 Detection and staining procedure	07
2.5 Counter stain procedure	07
Chapter 3 Limitations of procedure	08
3.1 Limitations	08
3.2 Interpretation of results	08
Chapter 4 References	10
Chapter 5 Probe specifications	11
Chapter 6 Trouble shooting guide	12
6.1 Introduction	12
6.2 No section or cells left on the slides	12
6.3 Weak or no staining on a suspected positive sample	13
6.4 Negative staining of the positive control	13
6.5 Positive staining of the negative control	14
6.6 Non-specific background staining	14
6.7 Cross hybridisation	14
Chapter 7 Language Reference Guides	15
7.1 English & German	15
7.2 French & Spanish	16
7.3 Dutch & Italian	17
7.4 Greek	18
Immaterial property information	20

Chapter 1 Introduction

1.1 Intended use

REMBRANDT® has been designed for the detection of specific DNA or RNA sequences by using the *in situ* Hybridisation (ISH) technique in paraffin embedded tissue sections, cytological specimens and frozen sections.

1.2 The ISH principle

ISH enables the detection of specific DNA or RNA sequences in histological and cytological specimens, without losing the often very essential morphological details. The principle of ISH is based on a “reaction” (= hybridisation) between a specifically labelled DNA or RNA sequence (= probe) and a DNA or RNA sequence present in the sample (= target). In case of matching sequences, a hybrid between the probe and target will be formed. Non-perfect matches are washed out by the stringency wash procedure (PanWash). The formed hybrids can easily be visualised by a specific staining procedure, i.e. substrate conversion by enzyme-conjugated antibodies. This conversion, i.e. the combination of NBT/BCIP and Alkaline Phosphatase (AP) conjugated anti-DIG or anti-BIO antibodies provided with this kit, will yield a detectable and coloured precipitation. The ISH technique is highly sensitive, specific, fast and easy to perform. Moreover, no radioactivity is involved. The reagents supplied with this kit are tailored to each other and therefore, REMBRANDT® is the ultimate user-friendly tool for performing ISH.

1.3 Controls

Use of both positive and negative controls is an essential part of the routine. To ensure that the ISH procedure is performed correctly and that observed positive and/or negative staining are specific, controls should be included in each experiment. This REMBRANDT® kit includes positive and negative control probes serving as a procedure control to be used on sections from the specimen under investigation. The positive control slides contain the desired target RNA and serve as a control for the specific probe. Additional control slides and probes are available from PanPath; please contact your local supplier.

1.4 Contents of a REMBRANDT® RISH & AP Detection Kit

Item label	description	Item (cap) colour	Item contents description	Item amount
DIGEST	PEPSIN POW	Black vial	: Pepsin digestion reagent	1 gram
DIGEST	PEPSIN DIL	Transparent vial	: Pepsin diluent (1N HCl solution)	15 mL
PROBE ²	Yellow/Purple vial	: Specific ¹ BIO or DIG labeled oligo probe(s)	0.8 mL
PROBE	+1 ² RISH	Pink vial	: RISH positive control oligo probe (BIO or DIG)	0.8 mL
PROBE	-1 ² RISH	Green vial	: RISH negative control oligo probe(BIO or DIG)	0.8 mL
CONJ ² AP	Red vial	: AP-conjugated anti-DIG or anti-BIO	15 mL
SUBS	NBT/BCIP	Blue vial	: NBT/BCIP substrate	15 mL
COUNT	NFR	Orange vial	: Nuclear Fast Red counterstain	15 mL
WASH	TBS	White pouches	: TBS buffer salt	2 pcs

1. Aljooze una gota de la solución de sonda por extracción y cubrir con una lamela
 2. Hidratiz
 3. Difundir verticalmente el lampón TBS sobre las lamelas
 4. Rinciez todas las lamelas con el lampón TBS
- PROTOCOLU DE DETECCION I DE COLOCACION
1. Distribuir 2-3 gotitas de conjugato (rojo) por specimen
 2. Immergier las lamelas en el lampón TBS
 3. Immergier las lamelas en el agua desionizada
 4. Aljooze 2-3 gotitas de substrato NBT/BCIP (bleu) a chaque echantillon et incuber dans l'obscurite
 5. Rinciez las lamelas con el agua desionizada o deionisee
 6. **Advertencia:** aljooze 2-3 gotitas de la solución colorante (orange)
 7. Rinciez las lamelas con el agua desionizada o deionisee
 8. Montez las secciones y examinez las lamelas al microscopio

3. Diluiz una alícuota de la solución de stock proteolítico con la solución diluida de HCl e incuizla: cada echantillon en 300-400 µL de la manera siguiente:
 diluion paraffine: 100x dans 0.1N HCl;
 aljooze 50 µL a 5 mL de HCl 0.01N
 diluion cytolologique: 25.000x dans 0.01N HCl;
 aljooze 4 µL a 100 µL de HCl 0.01N
2. Incuiz la solución de pepsina en alícuotas de 150 µL y congelaizlas a -20°C. Diluiz la solución de HCl 1N (solución transparente) en tanto que diluiz (transparente) según el uso que se requiere (parafina, citológica o congelada)

TEMPS D'INCUBATION

2-16 hrs à 56-60°C
2 x 10 min.
5 min.

DIGEST | PEP SIN POW
DIPEST | PEP SIN DL

- PROTOCOLU DE DETECCION II DE COLOCACION
1. Aljooze 2-3 gotitas de conjugato (rojo) a cada muestra
 2. Immergier las lamelas en el agua desionizada
 3. Immergier las lamelas en el agua desionizada
 4. Aljooze 2-3 gotitas de substrato NBT/BCIP (bleu) a chaque echantillon et incuber dans l'obscurite
 5. Rinciez las lamelas con el agua desionizada o deionisee
 6. **Advertencia:** aljooze 2-3 gotitas de solución de contraste (violeta naranja) a cada muestra
 7. Lavar las lamelas en agua desionizada
 8. Montar las portadas para su evaluación por microscopio

TEMPO DE INCUBAZIONE

2-16 horas a 56-60°C
2 x 10 minutos
5 minutos



1.8 Performance precautions

- Read all instructions before processing any assay.
- **DO NOT** use reagents beyond their expiry date.
- Allow all components to warm up to room temperature (20-25°C) before use.
- Homogenize probe solution before use.
- Avoid cross contamination of specimens.
- Work RNase-free directly after deparaffinisation until the hybridization step is completed.
- Wear gloves and treat glassware overnight at 200°C.
- **DO NOT** substitute a reagent with one from another manufacturer.
- When using treated glass slides other than those provided in the kit, specimens may fall off during the procedure.

1.9 Preparation of reagents in advance

Pepsin digestion reagent:

Dissolve the proteolytic reagent (black) in 4 mL of distilled or deionised water (upon receipt of the kit). Aliquot in portions of i.e.50 µL and store at -20°C.

Pepsin diluent:

Dilute the 1N HCl solution (transparent) to the application required concentration (paraffin sections 0,1 N; cytological and frozen preparations 0,01 N) with distilled or deionised water.

TBS buffer salt:

Dissolve 1 pouch in 1000 mL distilled or deionised water. Dissolve the salt completely and keep the buffer free from contamination.

1.10 Preparation of the proteolytic work solution

Prepare proteolytic work solution; 300 to 400 µL per section of 1 cm². Make fresh work solution just before use and discard non-used solution.

Paraffin sections:

dilute aliquoted proteolytic reagent 100x in 0.1N HCl, e.g. add 50 µL to 5 mL 0.1N HCl and mix.

Cytological specimens:

dilute aliquoted proteolytic reagent 25,000x in 0.01N HCl, e.g. add 4 µL to 100 mL 0.01N HCl and mix.

Frozen sections:

Dilute aliquoted proteolytic reagent 50,000x in 0.01N HCl, e.g. add 2 µL to 100 mL 0.01N HCl and mix.



6.5 Positive staining of the negative control

Possible causes	Remedies
■ Drying out of the section.	→ Incubate in a moisturised environment.
■ Washing procedure.	→ Make sure temperature is $37 \pm 2^\circ\text{C}$.
■ Contamination with positive control probe or specific probe.	→ Make sure that the positive control probe is the latest to be applied to the section.

6.6 Non-specific background staining

One should always bear in mind that the staining intensity and the level of background (or non-specific) staining may depend on the type of tissue used.

Possible causes	Remedies
■ Tissue section too thick.	→ Optimal thickness of the tissue is 4-6 μm .
■ Tissue crumbled.	→ Make sure tissue is stretched completely.
■ Deparaffinization.	→ Dewax series
■ Drying out of the section.	→ Incubate all procedure steps in a moisturised environment; prevent evaporation
■ Washing temperature.	→ Make sure temperature is $37 \pm 2^\circ\text{C}$.
■ Substrate incubation step too long.	→ Shorten incubation time with 5 minutes.
■ Endogenous peroxidase.	→ Inactivate endogenous peroxidase by incubating tissue sections in 3% $\text{H}_2\text{O}_2/\text{H}_2\text{O}$ for 15 minutes at room temperature prior to the digestion step.
■ Endogenous alkaline phosphatase.	→ Inactivate endogenous alkaline phosphatase by incubating sections in substrate solution to which 4 mg of levamisole is added.

6.7 Cross Hybridisation

One should always bear in mind that there is a possibility of cross hybridisation between related subtypes and that a patient can be infected with more than one subtype of a virus.

2.3 Hybridisation procedure (Rnase-free)*Hybridisation*

Homogenize probe solutions. Apply 1 drop or 20 μl of probe solution (yellow/purple) to each specimen and the positive control specimen. Apply 1 drop or 20 μl of the negative control probe (green) to each negative procedure control specimen and apply 1 drop or 20 μl of the positive control probe (pink) to each positive procedure control specimen. Cover all specimens with a cover slip (avoid air bubbles!). Transfer slides into a moist environment and incubate for 16 hours at 37°C (during the hybridisation the minimum temperature should be room temperature and the maximum temperature should be 37°C). Best results are obtained with prolonged incubation time (16 hours).

Washing

– Remove coverslips by submerging the slides in TBS buffer. Soak the slides until the coverslips fall off. Rinse the slides in TBS buffer for 10 minutes. Take the slides out, wipe off excess buffer and dry the edges using a lint-free cloth.

2.4 Detection and staining procedure

Apply 2-3 drops of AP-conjugate (red) to each specimen and transfer slides onto a 37°C heating block or slide warmer. Incubate for 30 minutes at 37°C . Tap off excess detection reagent and rinse the slides in TBS buffer. Soak 3x 1 minute in TBS buffer, while occasionally shaking the container. Transfer the slides into a container with distilled or deionised water and soak slides for 1 additional minute.

Take the slides out, wipe off excess of water and dry around the edges using a lint-free cloth. Ensure that the specimen on the slide is not disrupted. Apply 2-3 drops of NBT/BCIP substrate (blue) to each specimen and transfer the slides onto a 37°C heating block or slide warmer. Incubate in the dark for 5-15 minutes at 37°C (examine the colour development every 5 minutes microscopically). Tap off excess substrate solution and rinse the slides for 3x 1 minute in changes of distilled or deionised water. The slides are now ready to be mounted or counterstained.

2.5 Counterstain procedure

When a contrast colour is desired, the slides can be counterstained using Nuclear Fast Red (orange). Wipe off excess reagent and apply 2-3 drops of counterstain to each specimen. Incubate for 1 minute (longer incubation is possible and will yield stronger staining). Tap off excess counterstain and rinse the slides briefly in distilled or deionised water. Mount the slides by using an aqueous mounting medium. Interpret the results under the microscope.

Chapter 3 Limitations of Procedure

3.1 Limitations

- The Rembrandt DNA and RNA *in situ* Hybridisation and Detection kits are solely applicable for the detection of corresponding DNA or RNA which may be present in cell preparations (paraffin sections, frozen sections or cytological specimen).
- Appropriate medical decisions are only possible if the medical traceability is ensured. The product is intended for professional use as an aid in the diagnosis corresponding to the DNA RNA probes as supplied with the kit.
- Either human tissue sections or human cytological preparations may be used. Samples must be fixed in buffered formalin or alcohol. Sections should be cut at 4 µm thickness, glued to the glass slides with a bio-adhesive (e.g. organosilane), dried at room temperature, subsequently dried at 37 °C overnight, complete deparaffinisation in xylene and alcohol series and air dried. 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.
- 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.
- The performance of the ISH procedure is also affected by the sensitivity of the method and the DNA or RNA target load; in case the limit of the sensitivity is reached or when the target DNA or RNA load is too low, a false negative reaction may be the result.
- 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 morphological data. Negative results therefore do not rule out any possibility of a positive specimen.
- The Rembrandt test results are not to be relied on in case the sampling, sampling method, quality, sample preparation, reagents used, controls and procedure followed is not optimal.
- Therapeutic considerations based on the result of this test alone should not be taken. Positive results should be verified by other traditional diagnostic methods such as but not limited to clinical history, symptoms, as well as morphological data.
- The medical profession should be aware of risks and factors influencing the intensity, the absence or presence of probe signals which can not be foreseen when applying this test.
- The user should carefully consider the risk and use of sample material for this test in case the sample material does not contain sufficient or representative test material.
- Laboratory personnel performing the test should be knowledgeable and be able to interpret the test results.

3.2 Interpretation of the results

First, check the negative and positive controls that have been incubated with the test slides simultaneously:

- The negative control should be really negative, i.e. not show any localised colour precipitations. If the negative control could be interpreted as being positive, discard the results since no conclusions can be drawn.

6.3 Weak or no staining on a suspected positive sample

Possible causes	Remedies
■ Tissue fixation.	→ Only use buffered formalin fixative.
■ Deparaffinization.	→ Refresh dewax series.
■ Digestion.	→ Make sure correct concentration of pepsin is used. → Make sure digestion takes place at 37°C.
■ Interfering internal structures of probes.	→ In case of RISH procedures, denature sample at 60°C for 5 minutes before applying probe solution.
■ Hybridisation procedure.	→ Homogenize probe solution prior to applying probe on the section.
■ Washing temperature.	→ Make sure temperature is 37 ± 2°C.
■ Detection procedure.	→ Make sure temperature is 37 ± 2°C. → Make sure to incubate in the dark.
■ Low amount of target DNA.	→ Prolong hybridisation.
■ Colour precipitate rinsed away	→ Make sure that proper rinse and mounting media are used.

6.4 Negative staining of the positive control

Possible causes	Remedies
■ Deparaffinization	→ Re-fresh dewax series.
■ Positive control specimen incubated with positive control probe washed with PanWash. (Differentiation reagent)	→ Do not use PanWash (Differentiation reagent) on positive control specimen.
■ Interfering internal structures of probes.	→ In case of RISH procedures, denature sample at 60°C for 5 minutes before applying probe solution.
■ Detection procedure.	→ Make sure temperature is 37°C ± 2°C.

Chapter 6 Trouble Shooting Guide

6.1 Introduction

This Trouble Shooting Guide is intended to support you in obtaining optimal results with PanPath's REMBRANDT® *In Situ* Hybridisation and Detection kits.

In the next pages we inform you not only about possible causes and remedies for often occurring problems when performing ISH, but we also provide you with some tips given by experts on *In Situ* hybridisation that may be of help to you.

It is of course always possible that you encounter a problem which is not covered by this Trouble Shooting Guide, or that you still have doubts about your results. In such cases, please do not hesitate to contact your local supplier or PanPath directly. Since we consider your problem as our problem, we will do our utmost to find a proper solution.

6.2 No section or cells left on the slides

Possible causes	Remedies
■ Sample preparation.	→ Make sure samples are prepared according to protocol, the tissue is fixed in neutral buffered formalin and the slides are air dried well.
■ Tissue section too thin.	→ Optimal thickness of the tissue is 4-6 µm.
■ Wrong (side of) glass slide used.	→ Use only organosilane coated glass slides.
■ Pepsin concentration too high.	→ Make sure correct concentration of pepsin is used (depending on type of specimen).
■ Digestion step too long.	→ Reduce digestion time (15 minutes instead of 30 minutes) or digest at room temperature.
■ Coverslips removed with force.	→ Make sure that slides are soaked for at least 10 minutes in PBS.

- The positive control should show colour precipitations in conformity with the localisation of the target DNA or RNA. The colour should show the proper shade and must be clearly visible in the preferential cell/ tissue type and correspond to the target localisation.

In the test slides, start under low power magnification and focus on localisation and colour to see whether:

- The positivity (colour precipitation) observed is localised in the cell type preferred by the target.
- 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.

Product in combination with other devices

The Rembrandt *in situ* Hybridisation and Detection kits are intended for stand-alone usage. The *in vitro* diagnostic is intended to be used in combination with standard formalin fixed, paraffin embedded tissue blocks, standard tissue freezing, tissue sectioning (microtome), standard cytological preparation methods, hot plate(s), stove(s), incubation device(s), water bath(s), temperature and incubation time control(s), and other reagents (not supplied with this reagent) and a microscope. The combination has been tested and validated. Since the standard formalin fixed, paraffin embedded tissue blocks, standard tissue freezing, tissue sectioning (microtome), 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 fixation, embedding and dewaxing 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 the target load, which may influence the validation criteria.

Specifications of the RNA probes:

	EBER	kappa	lambda	Positive Control RNA	Negative Control RNA
Specificity	100%	100%	100%	100%	100%
Sensitivity	85%	85%	85%	95%	95%

Chapter 4 References

1. Autillo-Touati A. et al., HPV Typing by In Situ Hybridization on Cervical Cytologic Smears with ASCUS, *Acta Cytologica*, Vol. 42, p. 631-638, 1998.
2. Benkemoun A. et al., Evaluation of KREATECH In Situ Hybridization Kits for Detection of Human Papillomavirus DNA on Cervical Smears with "ASCUS", 3rd International Symposium "Impact of Cancer Biotechnology Diagnostic & Prognostic Indicators", Nice, France, October 1996. Accepted for publication in *Cancer Detection and Prevention*.
3. Botma H.J. et al., Differential In Situ Hybridization for Herpes Simplex Virus Typing in Routine Skin Biopsies, *Journal of Virological Methods*, Vol. 53, p. 37-45, 1995
4. Cooper K. et al., Human Papillomavirus DNA in Oesophageal Carcinomas in South Africa, *Journal of Pathology*, Vol. 175, p. 273-277, 1995.
5. Davidson B. et al., Angiogenesis in Uterine Cervical Intraepithelial Neoplasia and Squamous Cell Carcinoma: An Immunohistochemical Study, *International Journal of Gynecological Pathology*, Vol. 16, p. 335-338, 1997.
6. Davidson B. et al., CD44 Expression in Uterine Cervical Intraepithelial Neoplasia and Squamous Cell Carcinoma: An Immunohistochemical Study, *European Journal of Gynecology and Oncology*, Vol. XIX, no. 1, p. 46-49, 1998.
7. Davidson B. et al., Inflammatory Response in Cervical Intraepithelial Neoplasia and Squamous Cell Carcinoma of the Uterine Cervix, *Pathology Research and Practice*, Vol. 193, p. 491-495, 1997.
8. Gómez F. et al., Diagnosis of Genital Infection Caused by Human Papillomavirus Using In Situ Hybridization: The Importance of the Size of the Biopsy Specimen, *Journal of Clinical Pathology*, Vol. 48, p. 57-58, 1995.
9. Jing X. et al., Detection of Epstein-Barr Virus DNA in Gastric Carcinoma with Lymphoid Stroma, *Viral Immunology*, Vol. 10, No. 1, p. 49-58, 1997.
10. Sugawara I. et al., Detection of a Helicobacter Pylori Gene Marker in Gastric Biopsy Samples by Non-Radioactive In Situ Hybridization, *Acta Histochemica et Cytochemica*, Vol. 28, No. 3, p. 263-267, 1995.
11. Van den Brink W. et al., Combined β -Galactosidase and Immunogold/Silver Staining for Immunohistochemistry and DNA In Situ Hybridization, *Journal of Histochemistry and Cytochemistry*, Vol. 38, p. 325-329, 1990.
12. Yanai H. et al., Epstein-Barr Virus Infection in Non-Carcinomatous Gastric Epithelium, *Journal of Pathology*, Vol. 183, p. 293-298, 1997.
13. Yonezawa S. et al., MUC2 Gene Expression is Found in Non-invasive Tumors But Not in Invasive Tumors of the Pancreas and Liver: Its Close Relationship with Prognosis of the Patients, *Human Pathology*, Vol. 28, No. 3, p. 344-352, 1997.
14. Ziol M. et al., Virological and Biological Characteristics of Cervical Intraepithelial Neoplasia grade I with marked koilocytic Atypia, *Human pathology*, Vol 29, No. 10, p. 1068-1073, 1998.
15. Evans M. et al., Biotinyl-Tyramide-Based In Situ Hybridization Signal Patterns Distinguish Human Papilloma Virus Type and Grade of Cervical Intraepithelial Neoplasia, *Mod Pathol* 2002; 15(12):1339-1347
16. Hopman A. et al., Transition of high grade cervical intraepithelial neoplasia to micro-invasive carcinoma is characterized by integration of HPV 16/18 and numerical chromosome abnormalities, *J Pathol* 2004; 202:23-33
17. Hopman A. et al., Human papillomavirus integration: detection by in situ hybridization and potential clinical application, *J Pathol* 2004; 202:1-4
18. Hafkamp et al., A subset of head and neck squamous cell carcinomas exhibits integration of HPV 16/18 DNA and overexpression of p16^{INK4A} and p53 in the absence of mutations in p53 exons 5-8, *Int. J. Cancer*; 107(3):394-400

Chapter 5 Probe specifications

REMBRANDT® Biotin¹ and Digoxigenin^{1,2} labelled RNA probe specifications

CAT. NO.	LABEL	DNA PROBE SPECIFICATIONS		
		Description	Size	Region
A500P.0100 A500P.9900	BIO DIG	Epstein-Bar virus small RNA's probe (PROBE xxx EBER)*	30-mer oligonucleotide	mixture of 5 oligonucleotides complementary to Epstein-Bar encoded small RNA's
C601P.0100 C601P.9900	BIO DIG	Kappa light chain mRNA probe (PROBE xxx Kappa)*	30-mer oligonucleotide	mixture of 10 oligonucleotides complementary kappa light chain mRNA's
C602P.0100 C602P.9900	BIO DIG	Lambda light chain mRNA probe (PROBE xxx Lambda)*	30-mer oligonucleotide	mixture of 10 oligonucleotides complementary lambda light chain mRNA's
Q101P.0100 Q101P.9900	BIO DIG	Negative control probe for RNA (CONTROL – xxx RISH)*	26-mer oligonucleotide	1 oligonucleotide
Q152P.0100 Q152P.9900	BIO DIG	Positive control probe for RNA (CONTROL + xxx RISH)*	37-mer oligonucleotide	1 oligonucleotide complementary to Poly-A

* xxx = label (BIO or DIG)

- Contents : - clear vial, yellow cap = BIO labelled probe; 0.8 mL (25-40 assays)
- clear vial, purple cap = DIG labelled probe; 0.8 mL (25-40 assays)
- Format : ready to use
- Application : colorimetric detection of respective RNA in human specimen by *in situ* hybridisation (ISH)
- Detection limit : 10-30 pg by filter hybridisation
- Storage : refrigerated (2-8 °C); do not freeze
- Stability : until expiry date printed on label
- Precautions : - it is important to work RNase free in the period between deparaffinisation and hybridisation; wear gloves and treat glassware overnight at 200°C before use
- homogenise solutions before use
- avoid contact with eyes and skin; do not swallow

1 The probes in this product are labelled with the Universal Linkage System (ULS™). This product or the use of this product may be covered by one or more patents of KREATECH Biotechnology BV, including, but not restricted to, the following: EP 0539466; US 5,580,990; US 5,714,327; WO 92/01699; WO 96/35696; WO 98/15564.

2 Digoxigenin (DIG) labeling and detection is protected by international patents of Roche Molecular Biochemicals. This product is supplied under a license of Roche Molecular Biochemicals. This product or the use of this product may be covered by one or more patents of Roche Molecular Biochemicals, including the following: EP patent 0324 474 (granted); U.S. patent 5.354.657 (granted).