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GUIDE RÉFÉRENCE
DISH/RISH CONTROL PROBES

PRE-TRAITEMENT DES SECTIONS PARAFFINEES
TRAITEMENT PROTEOLYTIQUE

PROTOCOLE D'HYBRIDATION
1. Ajoutez une goutte ou 20 µl d'une solution de sonde par échantillon et couvrez avec une lamelle
2. Hybridez 2-16 hrs 37°C incubateur
3. Diffusez verticalement le tampon TBS sur les lamelles
4. Rincez toutes les lames avec le tampon TBS

Continuer avec:
PROTOCOLE DE DETECTION ET DE COLORATION

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HANDLEIDING
DISH/RISH CONTROL PROBES

SNIJDEN EN PLAKKEN VAN PARAFFINE COUPES
PROTEOLYTISCHE VOORBEHANDELING

HYBRIDISATIE PROCEDURE
1. Incubeer elk preparaat met probe reagens; 1 druppel of 20 µl en dek af met dekglaasje
2. Hybridiseer 2-16 uur bij 37°C stoof
3. Verwijder dekglaasje door preparaten in TBS buffer te dompelen
4. Spoel alle preparaten in TBS buffer

Ga verder met:
DETECTIE EN TEGENKLEURINGSPROCEDURE

GUIA DE REFERENCIA
DISH/RISH CONTROL PROBES

PRETRATAMIENTO DE LOS CORTES DE PARAFINA
TRATTAMIENTO PROTEOLITICO

PROTOCOLO DE HIBRIDACION
1. Añadir 1 gota o 20 µl de la solución de la sonda por muestra. Cubrir con un cubre.
2. Hibridizar 2-16 horas a 37ºC en un incubador
3. Retirar los cubres sumergiendo los portas en tampón TBS
4. Lavar todos los portas en tampón TBS

Continuar con:
PROTOCOLO DE DETECCION Y TINCION

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METODICA D'USO
DISH/RISH CONTROL PROBES

PRETRATTAMENTO DELLE SEZIONI IN PARAFFINA
TRATTAMENTO PROTEOLITICO

PROCEDIMENTO DI IBRIDAZIONE
1. Aggiungere 1 goccia o 20 µl di soluzione "probe" su ogni sezione. Coprire con coprivetrino.
2. Ibridizzare 2-16 ore a 37°C in incubatrice
3. Togliere il coprivetrino scia quando il vetrino in tampone TBS
4. Lavare tutti i vetrini in tampone TBS

Continue with:
PROCEDIMENTO DI DETEZIONE E COLORAZIONE

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

DATA SHEET-v4 CONTROL PROBES

PanPath

Product **Rembrandt® Biotin[1] and Digoxigenin[2] labelled DNA and RNA Control probes**
Code **QxxxP.xx00** (patents pending)

Technical specifications

Cat. No.	Label	DNA Probe specifications		
		Description	Size	Region
Q001P.0100 Q001P.9900	BIO DIG	Negative control probe for DNA (CONTROL – BIO DISH) Negative control probe for DNA (CONTROL – DIG DISH)	100-300 bp	pSP; 3.0 Kb
Q151P.0100 Q151P.9900	BIO DIG	Positive control probe for DNA (CONTROL + BIO DISH) Positive control probe for DNA (CONTROL + DIG DISH)	30-mer oligonucleotide	Mixture of six oligonucleotides complimentary to ALU repeats
Q101P.0100 Q101P.9900	BIO DIG	Negative control probe for RNA (CONTROL – BIO RISH) Negative control probe for RNA (CONTROL – DIG RISH)	26-mer oligonucleotide	1 oligonucleotide
Q152P.0100 Q152P.9900	BIO DIG	Positive control probe for RNA (CONTROL + BIO RISH) Positive control probe for RNA (CONTROL + DIG RISH)	37-mer oligonucleotide	1 oligonucleotide complementary to Poly-A

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 DNA and 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 until after the hybridisation; wear gloves and incubate laboratory materials overnight at 200 °C before use
- homogenise solutions before use
- avoid contact with eyes and skin; do not swallow

Related products
Probes and detection kits/reagents

Please contact your local supplier for further information.

1 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).
2 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.

Limitations of Procedure

Product Rembrandt® Biotin and Digoxigenin labelled DNA and RNA Control probes

- The REMBRANDT® DNA and RNA control probes are solely applicable for the detection of corresponding DNA and 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 and RNA probes as supplied.
- 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 um 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 been 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.

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.
- 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® DNA (DISH) and RNA (RISH) control probes 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), proteolytic-, detection- 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 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 the target load, which may influence the validation criteria.

Specifications of the RISH/DISH control probes:

	Positive control DNA	Negative control DNA	Positive Control RNA	Negative Control RNA
Specificity	100%	100%	100%	100%
Sensitivity	95%	95%	95%	95%

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REFERENCE GUIDE
DISH/RISH CONTROL PROBES

PRETREATMENT OF PARAFFIN SECTIONS
PROTEOLYTIC TREATMENT

HYBRIDIZATION PROCEDURE
1. Apply 1 drop or 20 µl of probe solution per specimen; cover with coverslip
2. Hybridize 2-16 hrs 37 °C incubator
3. Remove coverslips by soaking slides in TBS buffer
4. Wash all slides in TBS buffer

Continue with:
DETECTION AND STAINING PROCEDURE

PROBE .....

ANLEITUNG
DISH/RISH CONTROL PROBES

HERSTELLUNG VON PARAFFINSCHNITTEN
PROTEOLYTISCHE BEHANDLUNG

HYBRIDISIERUNGSPROZEDUR
1. Tropfen oder 20 µl der Sonde auf jedes Präparat geben und mit einem Deckglas abdecken
2. Hybridisieren 2-16 Stunden bei 37°C Ofen
3. Entfernen der Deckgläser durch Eintauchen in TBS Puffer
4. Präparate in TBS Puffer spülen

Verfolge mit:
DETEKTIONS-UND FÄRBEPROZEDUR