VIAL - LABEL

GUIDE REFERENCE DISH 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. Dénaturez 5 min. 95°C bloc chauffant
3. Hybridez 2-16 hrs 37°C incubateur
4. Diffusez verticalement le tampon TBS sur les lamelles
5. Ajoutez 5-6 gouttes de PanWash (blanc) 15 min. 37°C bloc chauffant à chaque échantillon à l'exception du contrôle positif
6. Rincez toutes les lames avec le tampon TBS

Continuer avec:
PROTOCOLE DE DETECTION ET DE COLORATION

HANDLEIDING DISH 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. Denatureer 5 min. bij 95°C hete plaat
3. Hybridiseer 2-16 uur bij 37°C stoof
4. Verwijder dekglaasje door preparaten in TBS buffer te dompelen
5. Incubeer elk preparaat met 15 min. bij 37°C hete plaat PanWash (wit); 5-6 druppels met uitzondering van de positieve controle
6. Spoel alle preparaten in TBS buffer

Ga verder met:
DETECTIE EN TEGENKLEURINGSPROCEDURE

PROBE .....

PANWASH GC% 50%

VIAL - LABEL

GUIA DE REFERENCIA DISH 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. Desnaturalizar 5 min. a 95ºC en una placa calefactora
3. Hibridizar 2-16 horas a 37ºC en un incubador
4. Retirar los cubres sumergiendo los portas en tampón TBS
5. Añadir 5-6 gotas de Pan Wash 15 minutos en un termobloque a 37ºC. (vial blanco) a cada muestra excepto al control positivo
6. Lavar todos los portas en tampón TBS

Continuar con:
PROTOCOLO DE DETECCION Y TINCION

METODICA D'USO DISH 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. Denaturare 5 min. su blocco riscaldante a 95°C
3. Ibridizzare 2-16 ore a 37°C in incubatrice
4. Togliere il coprivetrino scia quando il vetrino in tampone TBS
5. Aggiungere 5-6 gocce di PanWash 15 min. su blocco riscaldante a 37°C (bianco) su ogni sezione tranne sul controllo positivo
6. Lavare tutti i vetrini in tampone TBS

Continue with:
PROCEDIMENTO DI DETEZIONE E COLORAZIONE

PROBE .....

PANWASH GC% 50%

DATA SHEET-v4 DISH PROBES

PanPath

Product **DISH REMBRANDT® Biotin[1] and Digoxigenin[2] labelled DNA probes** (patents pending)
Code **AxxxP.xx00**

Technical specifications

Cat. No.	Label	DNA Probe specifications			
		Description	Size	Region	Vector
A100P.0100 A100P.9900	BIO DIG	Human papilloma virus HPV screening DNA probe (PROBE xxx panHPV)*	100-300 bp	mix of total genomes 7-8 Kb; containing the conserved HPV region	pBR322; 4.3 Kb and pSP; 3.0 Kb
A191P.0100 A191P.9900	BIO DIG	Human papilloma virus type 6/11 DNA probe (PROBE xxx HPV 6/11)*	100-300 bp	total genome 7.8 Kb HPV type 6 and 7.9 Kb HPV type 11	pSP; 3.0 Kb
A192P.0100 A192P.9900	BIO DIG	Human papilloma virus type 16/18 DNA probe (PROBE xxx HPV 16/18)*	100-300 bp	total genome 7.9 Kb HPV type 16 and 7.9 Kb HPV type 18	pSP; 3.0 Kb
A193P.0100 A193P.9900	BIO DIG	Human papilloma virus type 31/33 DNA probe (PROBE xxx HPV 31/33)*	100-300 bp	total genome 7.9 Kb HPV type 31 and 7.9 Kb HPV type 33	pBR322; 4.3 Kb and modified pSP; ≈ 4.0 Kb
A106P.0100 A106P.9900	BIO DIG	Human papilloma virus type 6 DNA probe (PROBE xxx HPV 6)*	100-300 bp	total genome 7.8 Kb HPV type 6	pSP; 3.0 Kb
A111P.0100 A111P.9900	BIO DIG	Human papilloma virus type 11 DNA probe (PROBE xxx HPV 11)*	100-300 bp	total genome 7.9 Kb HPV type 11	pSP; 3.0 Kb
A116P.0100 A116P.9900	BIO DIG	Human papilloma virus type 16 DNA probe (PROBE xxx HPV 16)*	100-300 bp	total genome 7.9 Kb HPV type 16	pSP; 3.0 Kb
A118P.0100 A118P.9900	BIO DIG	Human papilloma virus type 18 DNA probe (PROBE xxx HPV 18)*	100-300 bp	total genome 7.9 Kb HPV type 18	pSP; 3.0 Kb
A131P.0100 A131P.9900	BIO DIG	Human papilloma virus type 31 DNA probe (PROBE xxx HPV 31)*	100-300 bp	total genome 7.9 Kb HPV type 31	pBR322; 4.3 Kb
A133P.0100 A133P.9900	BIO DIG	Human papilloma virus type 33 DNA probe (PROBE xxx HPV 33)*	100-300 bp	total genome 7.9 Kb HPV type 33	modified pSP; ≈ 4.0 Kb
A200P.0100 A200P.9900	BIO DIG	Human Cytomegalo virus DNA probe (PROBE xxx CMV)*	100-300 bp	total genome 235 Kb	
A300P.0100 A300P.9900	BIO DIG	Epstein-Barr virus DNA probe (PROBE xxx EBV)*	100-300 bp	W fragment; 4.2 Kb	pDR720; 4.0 Kb
A400P.0100 A400P.9900	BIO DIG	Herpes simplex virus type 1/2 DNA probe (PROBE xxx HSV 1/2)*	100-300 bp	pSPM; 2.6 Kb	three HSV2 Sma I fragments; total ≈ 3.0 Kb
A600P.0100 A600P.9900	BIO DIG	Chlamydia trachomatis DNA probe (PROBE xxx CHLT)*	100-300 bp	endogenous sequences 7.2 Kb	pBluescript 2.9 Kb

* 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)
- white vial, white cap = matching PanWash; 15 mL
R013R.0000 for probes with GC % < 50%
R014R.0000 for probes with GC % = 50%

Format : ready to use
Application : colorimetric detection of respective DNA in human specimen by *in situ* hybridisation (ISH)
Purification : by size exclusion chromatography
Detection limit : 3-10 pg by filter hybridisation
Storage : refrigerated (2-8 °C); do not freeze
Stability : until expiry date printed on label
Precautions : - homogenise solutions before use
- avoid contact with eyes and skin; do not swallow

Related products
Universal ISH and detection kits product code: A000K.0105, A000K.0101, A000K.9905 or A000K.9901

Please contact your local supplier for further information.

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).

Limitations of Procedure

Product DISH REMBRANDT® DNA Probes

- The REMBRANDT® DNA probes are solely applicable for the detection of corresponding DNA 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 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 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 DNA probes:

	HPV[1]	CMV	HSV	EBV	CHLT
Specificity	95%	100%	100%	100%	100%
Sensitivity	85%	85%	85%	85%	85%

1 The specificity of the HPV DNA probes is 100% for the HPV species, but the different specific HPV probe sub-types may show some inter-type cross reactions.

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REFERENCE GUIDE DISH 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. Denature 5 min. 95°C heating block
3. Hybridize 2-16 hrs 37°C incubator
4. Remove coverslips by soaking slides in TBS buffer
5. Apply 5-6 drops of PanWash (white) to each specimen except the positive control 15 min. 37°C heating block
6. Wash all slides in TBS buffer

PROBE .....

PANWASH GC% ..50%

Continue with:
DETECTION AND STAINING PROCEDURE

ANLEITUNG DISH PROBES

HERSTELLUNG VON PARAFFINSCHNITTEN
PROTEOLYTISCHE BEHANDLUNG

HYBRIDISIERUNGSPROZEDUR

1. Tropfen oder 20 µl der Sonde auf jedes Präparat geben und mit einem Deckglas abdecken
2. Denaturieren 5 Min. bei 95°C Heizplatte
3. Hybridisieren 2-16 Stunden bei 37°C Ofen
4. Entfernen der Deckgläser durch Eintauchen in TBS Puffer
5. 5-6 Tropfen PanWash (Weiss) zu jedem Präparat geben (mit Ausnahme der Positivkontrolle) 15 Min. bei 37°C Heizplatte
6. Präparate in TBS Puffer spülen

Verfolge mit:
DETEKTIONS-UND FÄRBEPROZEDUR