HANDLEIDING
CONJUGATES

product code: R003R.0000
R004R.0000
R041R.0000
R042R.0000

VIAL - LABEL

METODICA D'USO
CONJUGATES

product code: R003R.0000
R004R.0000
R041R.0000
R042R.0000

SNIJDEN EN PLAKKEN VAN PARAFFINE COUPES
PROTEOLYTISCHE VOORBEHANDELING
HYBRIDISATIE PROCEDURE

Beginnen met *DETECTIE PROCEDURE*
1. Incubeer elk preparaat met conjugaat (rood); 2-3 druppels — 30 min. bij 37°C hete plaat — CONJ .....
2. Spoel de preparaten met TBS buffer
3. Spoel de preparaten met gedestilleerd/ gedeioniseerd water

Verder gaan met icubatie van het substraat en:

TEGENKLEURINGSPROCEDURE

PRETRATTAMENTO DELLE SEZIONI IN PARAFFINA
TRATTAMENTO PROTEOLITICO
PROCEDIMENTO DI IBRIDAZIONE

Start with *PROCEDIMENTO DI DETEZIONE*
1. Aggiungere ad ogni sezione 2-3 gocce di coniugato (rosso) — 30 min. su blocco riscaldante a 37°C
2. Sciacquare i vetrini in tampone TBS
3. Sciacquare i vetrini in acqua distillata/ deionizzata

Continue with incubare di soluzione substrato and:

PROCEDIMENTO DI COLORAZIONE

DATA SHEET-v4 CONJUGATES

Pan Path

Product **Anti Digoxigenin-Alkaline Phosphatase[1]**
Anti Digoxigenin-Horseradish Peroxidase[1]
Anti Biotin-Alkaline Phosphatase
Anti-Biotin-Horseradish Peroxidase

Code **R003R.0000, R004R.0000, R041R.0000, R042R.0000**

Technical specifications

Cat. No.	Description	Contents	Format	Appearance	Activity
R003R.0000	Anti digoxigenin-alkaline phosphatase conjugate ; Fab fragments; (CONJ DIG-AP)	15 ml (100 tests), clear vial, red cap	Ready to use	Clear liquid; colourless	Approx. 2 units/mL; 1 unit hydrolyses 1 umole of p-nitriphenyl phosphate per minute at pH 9.8 at 37 °C
R004R.0000	Anti digoxigenin-horseradish peroxidase conjugate; Fab fragments; (CONJ DIG-HRP)	15 ml (100 tests), clear vial, red cap	Ready to use	Clear liquid; colourless	Approx. 2 units/mL; 1 unit forms 1 mg purpurogallin in 20 seconds from pyrogallol at pH 6.0 at 20 °C
R041R.0000	Anti biotin-alkaline phosphatase conjugate; Fab fragments; (CONJ BIO-AP)	15 ml (100 tests), clear vial, red cap	Ready to use	Clear liquid; colourless	Approx. 2 units/mL; 1 unit hydrolyses 1 umole of p-nitriphenyl phosphate per minute at pH 9.8 at 37 °C
R042R.0000	Anti biotin-horseradish peroxidase conjugate; Fab fragments; (CONJ BIO-HRP)	15 ml (100 tests), clear vial, red cap	Ready to use	Clear liquid; colourless	Approx. 2 units/mL; 1 unit forms 1 mg purpurogallin in 20 seconds from pyrogallol at pH 6.0 at 20 °C

Application : conjugate for colorimetric detection of digoxigenin (dig) or biotin (bio) labelled probes by *in situ* hybridisation (ISH)
Performance : 3.2 pg nucleic acids (labelled), checked by direct spot blot
Purification : affinity chromatography
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
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).

Limitations of Procedure

Product Conjugates

- The REMBRANDT® Conjugates are solely applicable for the detection and visualisation of hybridised Biotin or Digoxigenin labelled DNA and RNA probes in ISH, 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 by *in situ* hybridisation.
- 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, incubation temperatures, all other reagents (i.e. deparaffinisation reagents, proteolytic treatment, probes etc.) used in the procedure 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. Moreover, diagnosis should also take the clinical history, symptoms, as well as morphological data into consideration. 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, other 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® Conjugates 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), other needed reagents (but not supplied with this reagent) for ISH 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 needed reagents such as but not limited to proteolytic reagents, labelled probes, substrate 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; since the Conjugates can be used for all targets labelled with biotin or digoxigenin and the results also depend on the target load, the validation criteria may vary.

References

1. *Chalet, L. and Wolf, F., Arch. Biochem. Biophys., 106, 1 (1964).*
2. *Green, N.M., Meth. Enzymol., 18A, 418 (1970).*

REFERENCE GUIDE
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PRETREATMENT OF PARAFFIN SECTIONS
PROTEOLYTIC TREATMENT
HYBRIDIZATION PROCEDURE

Start with *DETECTION PROCEDURE*
1. Apply 2-3 drops of the conjugate (red) to each specimen — 30 min. on a 37°C heating block
2. Soak slides in TBS buffer
3. Soak slides in distilled/deionised water

Continue with substrate incubation and proceed with:

STAINING PROCEDURE

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ANLEITUNG
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HERSTELLUNG VON PARAFFINSCHNITTEN
PROTEOLYTISCHE BEHANDLUNG
HYBRIDISIERUNGSPROZEDUR

Anfangen mit *DETEKTIONSPROZEDUR*
1. 2-3 Tropfen Konjugat (Rot) auf jedes Präparat geben — 30 Min. bei 37°C Heizplatte
2. Präparate in TBS Puffer spülen
3. Präparate mit destilliertem/ deionisiertem Wasser spülen

Verfolge mit Substrat Inkubation und mit:

FÄRBEPROZEDUR

GUIDE RÉFÉRENCE
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PRE-TRAITEMENT DES SECTIONS PARAFFINEES
TRAITEMENT PROTEOLYTIQUE
PROTOCOLE D'HYBRIDATION

Commencer avec *PROTOCOLE DE DETECTION*
1. Distribuez 2-3 gouttes de conjugant (rouge) par spécimen — 30 min. 37°C bloc chauffant
2. Immergez les lames dans le tampon TBS
3. Immergez les lames dans de l'eau distillée ou deionisée

Continuer avec incubez la solution substrate et:

PROTOCOLE DE COLORATION

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GUIA DE REFERENCIA
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PRETRATAMIENTO DE LOS CORTES DE PARAFINA
TRATAMIENTO PROTEOLITICO
PROTOCOLO DE HIBRIDACION

Comenzar con *PROTOCOLO DE DETECCION*
1. Añadir 2-3 gotas del conjugado (vial rojo) a cada muestra — 30 min. en un termobloque a 37ºC
2. Sumergir los portas en tampón TBS
3. Sumegir los portas en agua desionizada

Continuar con incubar de solución de substrato más:

PROTOCOLO DE TINCION