HANDLEIDING

SUBSTRATES & SUBSTRATE BUFFER

product code: R007R.0000
R008R.0000
R010R.0000

SNIJDEN EN PLAKKEN VAN PARAFFINE COUPES
PROTEOLYTISCHE VOORBEHANDELING
HYBRIDISATIE PROCEDURE

Beginnen met *DETECTIE PROCEDURE* (conjugaat) en verder met A of B:

(A) AEC substraat

1. Bereid AEC (blauw) werkoplossing volgens onderstaande tabel

Aantal preparaten	Aantal druppels AEC substraat	Volume AEC buffer
1-13	4	2 mL
14-26	8	4 mL
27-39	12	6 mL
40-52	16	8 mL

2. Incubeer elk preparaat met de werkoplossing; 2-3 druppels en incubeer in donker
3. Spoel de preparaten met gedestilleerd/ gedeioniseerd water

(B) NBT/BCIP substraat

4. Incubeer elk preparaat met NBT/BCIP substraat (blauw); 2-3 druppels en incubeer in donker — 5-15 min. bij 37°C hete plaat
5. Incubeer elk preparaat met de werkoplossing; 2-3 druppels en incubeer in donker
6. Spoel de preparaten met gedestilleerd/ gedeioniseerd water

Verder gaan met:
TEGENKLEURINGS PROCEDURE

VIAL - LABEL

SUBS	AEC
BUFF	AEC

SUBS	NBT/BCIP

METODICA D'USO

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PRETRATTAMENTO DELLE SEZIONI IN PARAFFINA
TRATTAMENTO PROTEOLITICO
PROCEDIMENTO DI IBRIDAZIONE

Start with *PROCEDIMENTO DI DETEZIONE* (conjugato) and proceed with A or B:

(A) AEC substrato

1. Preparare la soluzione di lavoro dell'AEC (blu) come segue:

No. di sezioni	No. di gocce di substrato AEC	Volume di tampone AEC
1-13	4	2 mL
14-26	8	4 mL
27-39	12	6 mL
40-52	16	8 mL

2. Aggiungere ad ogni sezione 2-3 gocce di soluzione di lavoro e incubare al buio
3. Togliere l'eccesso di soluzione substrato e lavare i vetrini in acqua distillata/deionizzata

(B) NBT/BCIP substrato

1. Aggiungere ad ogni sezione 2-3 gocce di substrato NBT/BCIP (blu) e incubare al buio — 5-15 min. su blocco riscaldante a 37°C
2. Aggiungere ad ogni sezione 2-3 gocce di soluzione di lavoro e incubare al buio
3. Togliere l'eccesso di soluzione substrato e lavare i vetrini in acqua distillata/deionizzata

Continue with:
PROCEDIMENTO DI COLORAZIONE

DATA SHEET-V4 PRECIPITATING SUBSTRATES

PanPath

Product **AEC substrate, NBT/BCIP substrate, AEC substrate buffer**
Code **R007R.0000, R008R.0000, R010R.0000**

Technical specifications

Cat. No.	Description	Contents	Format	Appearance	pH	Result
R007R.0000	3-amino-9-ethylcarbazole HRP substrate for use in conjunction with AEC buffer (SUBS AEC)*	2 mL (100 tests), clear vial, blue cap	Concentrated in dimethylformamid	Dark brown liquid	NA	Reddish-brown precipitate
R010R.0000	AEC buffer solution containing hydrogen peroxidase for use in conjunction with AEC substrate (BUFF AEC)*	15 mL (100 tests), white vial, blue cap	Pre-diluted	Clear liquid; colourless	4.8 ±0.2	Reddish-brown precipitate
R008R.0000	Nitroblue tetrazolium salt and 5-bromo-4-chloro-3-indolyl phosphate AP substrate (SUBS NBT/BCIP)	15 mL (100 tests), white vial, blue cap	Ready to use	Clear liquid, yellow	9.0±0.2	Indigo precipitate

*Directions for preparing AEC working solution:

# of specimens	# of drops of AEC substrate	Vol. of AEC buffer
1-13	4	2 mL
14-26	8	4 mL
27-39	12	6 mL
40-52	16	8 mL

Application : substrate for colorimetric detection of conjugates by in situ hybridisation (ISH)
Performance : 3.2 pg DNA (labelled and conjugated), checked by direct spot blot
Storage : refrigerated (2-8 °C) protected from light
Stability : until expiry date printed on label
Precautions : - AEC is a potential carcinogen
- homogenise solutions before use
- avoid contact with eyes and skin; do not swallow

Related products

Please contact your local supplier for further information.

Limitations of Procedure

Product Precipitating Substrates

- The REMBRANDT® Precipitating Substrates are solely applicable for the visualisation of conjugated antibodies which are used for the detection 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® Precipitating Substrates 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, conjugates 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 Precipitating Substrates can be used for all targets labelled with AP or HRP conjugates and the results also depend on the target load , the validation criteria may vary.

References

1. *Graham, R.C., et al., J. Histochem. Cytochem., 13, 150 (1965).*
2. *Kaplow, L.S., am J. Clin. Path., 63, 451 (1974).*
3. *Blake, M., Anal. Biochem., 136, 175 (1984).*
4. *Horowitz, J., et al., J. Med. Chem., 9, 447 (1966).*

REFERENCE GUIDE

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PRETREATMENT OF PARAFFIN SECTIONS
PROTEOLYTIC TREATMENT
HYBRIDIZATION PROCEDURE

Start with *DETECTION PROCEDURE* (conjugate) and proceed with A or B:

(A) AEC substrate

1. Prepare AEC (blue) work solution according the following table

# of specimens	# of drops of AEC substrate	Vol. of AEC buffer
1-13	4	2 mL
14-26	8	4 mL
27-39	12	6 mL
40-52	16	8 mL

2. Apply 2-3 drops of the work solution to each specimen and incubate in dark
3. Tap off excess substrate solution and wash slides in distilled/deionised water

(B) NBT/BCIP substrate

1. Apply 2-3 drops of NBT/BCIP substrate(blue) to each specimen and incubate in dark — 5-15 min. 37°C heating block
2. Apply 2-3 drops of the work solution to each specimen and incubate in dark
3. Tap off excess substrate solution and wash slides in distilled/deionised water

Continue with:
STAINING PROCEDURE

VIAL - LABEL

SUBS | AEC
BUFF | AEC

SUBS | NBT/BCIP

ANLEITUNG

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HERSTELLUNG VON PARAFFINSCHNITTEN
PROTEOLYTISCHE BEHANDLUNG
HYBRIDISIERUNGSPROZEDUR

Anfangen mit *DETEKTIONSPROCEDUR* (Konjugat) und verfolge mit A oder B:

(A) AEC Substrat

1. AEC (Blau) Gebrauchslösung nach nachfolgendem Schema vorbereiten:

Praeparate-Anzahl	Tropfen der AEC Substratloesung	Vol. AEC buffer
1-13	4	2 mL
14-26	8	4 mL
27-39	12	6 mL
40-52	16	8 mL

2. 2-3 Tropfen der Gebrauchslösung zu jedem Präparat geben Im dunkeln inkubieren
3. In destilliertem/deionisiertem Wasser spülen

(B) NBT/BCIP Substrat

1. 2-3 Tropfen des NBT/BCIP-Substrats (Blau) auf jedes Präparat geben Im dunkeln inkubieren — 5-15 Min. bei 37°C Heizplatte
2. 2-3 Tropfen der Gebrauchslösung zu jedem Präparat geben Im dunkeln inkubieren
3. In destilliertem/deionisiertem Wasser spülen

Verfolge mit:
FÄRBEPROZEDUR

GUIDE RÉFÉRENCE

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PRE-TRAITEMENT DES SECTIONS PARAFFINÉES
TRAITEMENT PROTEOLYTIQUE
PROTOCOLE D'HYBRIDATION

Commencer avec *PROTOCOLE DE DÉTECTION* (conjugant) et avec A ou B:

(A) AEC substrat

1. Préparez la solution d'usage AEC (bleu) à partir du tableau ci-dessous:

No. Echantillon	No. gouttes de AEC substrate	Vol. de solution tampon AEC
1-13	4	2 mL
14-26	8	4 mL
27-39	12	6 mL
40-52	16	8 mL

2. Ajoutez 2-3 gouttes de la solution d'usage par spécimen et incubez dans l'obscurité
3. Rincez les lames avec de l'eau distillée ou deionisée

(B) NBT/BCIP substrat

1. Ajoutez 2-3 gouttes du substrat NBT/BCIP (bleu) à chaque échantillon et incubez dans l'obscurité — 5-15 min. 37°C bloc chauffant
2. Ajoutez 2-3 gouttes de la solution d'usage par spécimen et incubez dans l'obscurité
3. Rincez les lames avec de l'eau distillée ou deionisée

Continuer avec :
PROTOCOLE DE COLORATION

VIAL - LABEL

SUBS | AEC
BUFF | AEC

SUBS | NBT/BCIP

GUIA DE REFERENCIA

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PRETRATAMIENTO DE LOS CORTES DE PARAFINA
TRATAMIENTO PROTEOLITICO
PROTOCOLO DE HIBRIDACION

Commenzar con *PROTOCOLO DE DETECCION* (conjugado) más con A u B:

(A) AEC substrato

1. Preparar el AEC a la solución de trabajo de acuerdo con la siguiente tabla:

Muestras	Gotas de substrato AEC	Volumen de tampón AEC
1-13	4	2 ml
14-26	8	4ml
27-39	12	6ml
40-52	16	8ml

5. Añadir 2-3 gotas de la solución de trabajo a cada muestra e incubar en oscuridad
6. Eliminar el exceso de solución de substrato y lavar los portas en agua destilada o desionizada

(B) NBT/BCIP substrato

1. Añadir 2-3 gotas del substrato NBT/BCIP (vial azul) a cada muestra e incubar en oscuridad — 5-15 minutos a 37ºC en un termobloque
2. Añadir 2-3 gotas de la solución de trabajo a cada muestra e incubar en oscuridad
3. Eliminar el exceso de solución de substrato y lavar los portas en agua destilada o desionizada

Continuar con:
PROTOCOLO DE TINCION