HANDLEIDING
DIGESTION REAGENTS

product code: R011R.0000
R018R.0000

SNIJDEN EN PLAKKEN VAN PARAFFINE COUPES

PROTEOLYTISCHE VOORBEHANDELING

1. Los het pepsine poeder (zwart) in 4 mL gedestilleerd/gedeioniseerd water op, verdeel in 150 µL porties en bewaar bij -20°C.

2. Verdun het 1N HCl pepsine oplosmiddel (transparant) tot de concentratie die voor de applicatie nodig is (paraffine, cytologie of vries coupe; zie 3)
3. Verdun de proteolytische stock oplossing in het verdunde HCl en incubeer elk preparaat met 300-400 µL:
   paraffine: 100x in 0.1N HCl;
   voeg 50 µL aan 5 mL 0.1N HCl toe — 30 min. bij 37°C hete plaat
   cytologie: 25,000x in 0.01N HCl;
   voeg 4 µL an 100 mL 0.01N HCl toe — 10 min. bij 37°C hete plaat
   vries: 50,000x in 0.01N HCl;
   voeg 2 µL aan 100 mL 0.01N HCl toe — 10 min. bij 37°C hete plaat
4. Overmaat van de proteolytische werk-oplossing weggooien(altijd vers bereiden)
5. Ontwater de preparaten in oplopende alcoholreeks en luchtdrogen

Verder gaan met:

HYBRIDISATIE PROCEDURE
DETECTIE EN TEGENKLEURINGSPROCEDURE

VIAL - LABEL

DIGEST	PEPSIN.....

METODICA D'USO
DIGESTION REAGENTS

product code: R011R.0000
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PRETRATTAMENTO DELLE SEZIONI IN PARAFFINA

TRATTAMENTO PROTEOLITICO

1. Seguendo le istruzioni del kit, sciogliere la pepsina in polvere (nera) in 4 mL di acqua distillata/deionizzata, quindi preparare aliquote di 150 µL e congelare a -20°C.
2. Diluire la pepsina 1N HCl (trasparente) diluente sino a raggiungere la concentrazione richiesta dall'applicazione (Paraffinati, Citologici o Congelati; vedasi punto 3)
3. Diluire la soluzione proteolitica scongelata in HCl diluito secondo lo schema seguente (Incubare ogni sezione con 300-400 µL):
   Paraffinati: 100x in 0.1N HCl;
   aggiungere 50 µL a 5 mL 0.1N HCl — 30 min. su blocco riscaldante a 37°C
   Citologici: 25'000x in 0.01N HCl;
   aggiungere 4 µL a 100 mL 0.01N HCl — 10 min. su blocco riscaldante a 37°C
   Congelati: 50'000x in 0.01N HCl;
   aggiungere 2 µL a 100 mL 0.01N HCl — 10 min. su blocco riscaldante a 37°C
4. Gettare l'eccedenza della soluzione proteolitica
5. Disidratare le sezioni in etanolo puro ed asciugare all'aria

Continue with:

PROCEDIMENTO DI IBRIDAZIONE
PROCEDIMENTO DI DETEZIONE E COLORAZIONE

DATA SHEET-V4 DIGESTION REAGENTS

Pan Path

Product **Pepsin digestion reagent, Pepsin diluent**
Code **R011R.0000, R018R.0000**

Technical specifications

Cat. No.	Description	Contents	Format	Appearance	pH
R011R.0000	Pepsin digestion reagent, to be used in combination with Pepsin diluent (DIGEST PEPSIN POW)	1 g lyophilised powder, clear vial, black marked cap	To be diluted and mixed	Powder	NA
R018R.0000	Pepsin diluent, 1 N HCl, to be used in combination with Pepsin digestion reagent (DIGEST PEPSIN DIL)	15 mL, clear vial, clear cap	To be diluted and mixed	Clear liquid	< 1

Recommended use for proteolytic treatment:

1. Dissolve pepsin powder (black) in 4 mL distilled/deionised water, aliquot in 150 µL batches and freeze at -20°C (proteolytic stock).
2. Dilute the 1N HCl pepsin diluent (transparent) to the application required concentration (paraffin, cytological or frozen; see 3)
3. Dilute thawed proteolytic stock solution in diluted HCl
   paraffin: 100x in 0.1N HCl
   i.e. add 50 µL to 5 mL 0.1N HCl
   cytological: 25,000x in 0.01N HCl
   i.e. add 4 µL to 100 mL 0.01N HCl
   frozen: 50,000x in 0.01N HCl
   i.e. add 2 µL to 100 mL 0.01N HCl

Application : digestion reagents for use in *in situ* hybridisation (ISH)
Performance : - NFR for use in combination with NBT/BCIP substrate to contrast the indigo precipitate
- MG for use in combination with AEC substrate to contrast the reddish-brown precipitate
Storage : refrigerated (2-8 °C) protected from light; do not freeze
Stability : until expiry date printed on label
Precautions : - homogenise solutions before use
- irritant, avoid contact with eyes and skin; do not swallow

Related products
Please contact your local supplier for further information.

Limitations of Procedure

Product Digestion reagents

- The REMBRANDT® Digestion reagents are solely applicable in the digestion procedure step in ISH.
- 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® Digestion reagents 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 labelled probes, 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; since the Digestion reagents can be used for all ISH applications and are also depending on the target load, the validation criteria may vary.

References

1. Anson, M., J. Gen. Physiol., 22, 79 (1938).

REFERENCE GUIDE
DIGESTION REAGENTS

product code: R011R.0000
R018R.0000

PRETREATMENT OF PARAFFIN SECTIONS

PROTEOLYTIC TREATMENT

1. Dissolve pepsin powder (black) in 4 mL distilled/deionised water, aliquot in 150 µL batches and freeze at -20°C.
2. Dilute the 1N HCl pepsin diluent (transparent) to the application required concentration (paraffin, cytological or frozen; see 3)
3. Dilute thawed proteolytic stock solution in diluted HCl and incubate each specimen with 300-400 µL:
   paraffin: 100x in 0.1N HCl;
   add 50 µL to 5 mL 0.1N HCl 30 min. on 37°C heating block
   cytological: 25,000x in 0.01N HCl;
   add 4 µL to 100 mL 0.01N HCl 10 min. on 37°C heating block
   frozen: 50,000x in 0.01N HCl;
   add 2 µL to 100 mL 0.01N HCl 10 min. on 37°C heating block
4. Discard excess proteolytic work solution
5. Dehydrate slides in graded ethanol and air dry

Continue with:

HYBRIDIZATION PROCEDURE
DETECTION AND STAINING PROCEDURE

VIAL-LABEL
DIGEST PEPSIN.....

ANLEITUNG
DIGESTIONS REAGENTS

product code: R011R.0000
R018R.0000

HERSTELLUNG VON PARAFFINSCHNITTEN

PROTEOLYTISCHE BEHANDLUNG

1. Pepsin Pulver (Schwarz) in 4 mL destilliertem/deionisiertem Wasser lösen, in 150 µL Portionen aliquotieren und bei -20°C aufbewahren
2. 1N HCl Pepsin-Lösungsmittel (Transparent) auf die Konzentration verdünnen, die für die entsprechende Anwendung notwendig ist (Paraffinschnitt, Zytologisches Präparat oder Gefrierschnitt; siehe 3)
3. Verdünne die aufgetaute proteolytische Lösung in verdünntem HCl; jedes Präparat mit 300-400 µL inkubieren.
   Paraffinschnitt: 100x in 0.1N HCl;
   50 µL zu 5 mL 0.1N HCl geben 30 Min. bei 37°C Heizplatte
   Zytologisches Praeparat: 25,000x in 0.01N HCl;
   4µL zu 100 mL 0.01N HCl geben 10 Min bei 37°C Heizplatte
   Gefrierschnitt: 50,000x in 0.01N HCl;
   2 µL zu 100 mL 0.01N HCl geben 10 Min. bei 37°C Heizplatte
4. Überschüssige proteolytische Lösung verwerfen
5. Schnitte in Ethanolreihe entwässern und lufttrocknen

Verfolge mit:

HYBRIDISIERUNGSPROZEDUR
DETEKTIONS-UND FÄRBEPROZEDUR

VIAL-LABEL
DIGEST PEPSIN.....

GUIDE RÉFÉRENCE
DIGESTION REAGENTS

product code: R011R.0000
R018R.0000

PRE-TRAITEMENT DES SECTIONS PARAFFINEES

TRAITEMENT PROTEOLYTIQUE

1. Dissolvez la poudre de pepsine (noire) dans 4 ml d'eau distillée ou deinonisée, divisez la solution en aliquotes de 150 µl et congelez les (-20°C).
2. Diluez la solution HCl 1N (solution de pepsine en tant que diluant; transparent) selon l'usage préféré (paraffiné, cytologique ou congelé)
3. Diluez une aliquote de la solution du stock proteolytique avec la solution diluée de HCl et incubez chaque échantillon dans 300-400 µl de la manière suivante:
   dilution paraffinée: 100x dans 0.1N HCl;
   ajouter 50 µl à 5 ml de HCl 0.01N 30 min. 37°C bloc chauffant
   dilution cytologique: 25,000x dans 0.01N HCl;
   ajouter 4 µl à 100 ml HCl 0.01N 10 min. 37°C bloc chauffant
   dilution congelée: 50,000x dans 0.01N HCl;
   ajouter 2 µl à 100 ml de HCl 0.01N 10 min. 37°C bloc chauffant
4. Rincez avec de l'eau distillée ou deionisée
5. Deshydratez les lames dans une série d'éthanol et laissez sécher à l'air 3 x 1 min.

Continuer avec:

PROTOCOLE D'HYBRIDATION
PROTOCOLE DE DETECTION ET DE COLORATION

VIAL-LABEL
DIGEST PEPSIN.....

GUIA DE REFERENCIA
DIGESTION REAGENTS

product code: R011R.0000
R018R.0000

PRETRATAMIENTO DE LOS CORTES DE PARAFINA

TRATAMIENTO PROTEOLITICO

1. A la recepción del kit disolver la pepsina en polvo (vial negro) en 4 ml de agua destilada/desionizada, hacer alícuotas de 150 µl y congelar a –20ºC.
2. Diluir el diluyente de la pepsina (vial transparente) ClH 1N a la concentración requerida para la aplicación (parafina, citología o congelación; ver 3)
3. Diluir la solución proteolítica stock descongelada en ClH diluido e incubar cada muestra con 300-400 µl:
   Parafina: 100X en ClH 0.1N;
   añadir 50 µl a 5 ml de ClH 0.1N 30 min. en un termobloque a 37ºC.
   Citología: 25.000X en ClH 0.01N;
   añadir 4 µl a 100ml de ClH 0.01N 10 min. en un termobloque a 37ºC
   Congelación: 50.000X en ClH 0.01N;
   añadir 2 µl a 100 ml de ClH 0.01N 10 min. en un termobloque a 37ºC
4. Eliminar el exceso de solución proteolítica a la dilución de trabajo
5. Deshidratar los portas en soluciones alcohólicas crecienbtes y secar al aire

Continuar con:

PROTOCOLO DE HIBRIDACION
PROTOCOLO DE DETECCION Y TINCION