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siehe unsere Liefer- und Versandbedingungen

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- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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User's Manual and Instructions

IsHyb In Situ Hybridization (ISH) Kit

Catalog Number: K2191020, K2191050

Introduction

In situ hybridization (ISH) is a powerful method for gene expression analysis. We offer a high-quality, fully customizable in situ hybridization kit for gene related studies. The IsHyb In Situ Hybridization Kit provides the reagents, including hybridization buffer, NBT/BCIP color development solution and washing buffer solutions, for pre-hybridization, hybridization, and post hybridization treatments.

Features

- microRNA detection
- Non-isotopic, colorimetric detection of gene expression
- RNase-free, ISH-optimized reagents
- Can be used with either oligonucleotide or ribonucleotide probes
- Tested by Digoxingenin labeled oligonucleotide probes
- Optimization of ISH assay conditions
- ISH detection in tissue section and/or tissue microarray slides
- Full technical support

Applications

- Diagnostic determination and clinical decision-making
- Gene mutation studies
- Protein expression and function analysis

Description

The ISH kit provides researchers a method doing in situ hybridization with nonradioactive probes. Compared with radioactive methods, the sensitivity of this kit is the same while the procedure is simplified. This kit is suitable for digoxingenin labled probes combined with an alkaline phosphatase color development system. Using this kit, all disadvantages of working with radioactive materials can be avoided.

Quality control

In order to ensure the quality of IsHyb ISH kit, a pre-test procedure is performed for each lot of the kits. The positive result is detected with a digoxingenin labeled Has-mirRNA 122a cDNA probe on a previous positive frozen tissue panel slide.

Contents

Item	K2191020 Amount	K2191020 Part No.	K2191050 Amount	K2191050 Part No.
Prehybridization solution	2.0 ml	K2191020 -1	5 ml	K2191050 -1
Hybridization solution	2.0 ml	K2191020 -2	5 ml	K2191050 -2
NBT	120 µl	K2191020 -3	300 µl	K2191050 -3
BCIP	60 µl	K2191020 -4	150 µl	K2191050 -4
20 x SSC buffer	20.0 ml	K2191020 -5	50 ml	K2191050 -5
10x Alkaline phosphatase buffer	20.0 ml	K2191020 -6	50 ml	K2191050 -6
Anti-Dig Antibody	10 µl	K2191020 -7	25 µl	K2191050 -7
100 x Blocking solution	0.1 ml	K2191020 -8	0.25 ml	K2191050 -8

K2191020 isdesigned for hybridization of 20 slides, K2191050 isfor 50 slides.

Buffer required but not included in the kit 1x PBS solution, pH 7.4: 81 ml 0.2 M Na_2HPO_4 19 ml 0.2 M NaH_2PO_4 18 g NaCl Add distilled water to 2,000 ml.

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Protocol

Preparation of frozen sections or paraffin sections

A: Pre-Treatment of Sections

1 Warm frozen slides to room temperature and dry at 50ºC for 15 minutes

2 Paraffin slides: deparaffinization and rehydration through the following procedure:

1) Deparaffinization: Soak tissue slides with xylene twice, each time for 15 minutes 2) Rehydration: Let slides go through graded ethanol: 100%I, 100%II, 95%, 90%, 80%, 70%, and distilled water in the end. 5 minutes for each concentration.

Note: Bakingtheparaffinsectionsat60ºCfor1-2hoursisrecommendedif tissues drop off during following procedure

B: Hybridization

1 Fix the slides with 4% paraformaldehyde in DEPC-PBS at room temperature for 20 minutes.

2 Wash the slides twice with DEPC-PBS at room temperature for 5 minutes.

3 Treat the slides with 10 µ g/ml Proteinase K at 37ºC for 8-15 minutes.

4 Wash the slides once in DEPC-PBS at room temperature for five minutes. Fix them again with 4% paraformaldehyde in DEPC-PBS for 15 minutes. Rinse the slides once with DEPC-water.

5 Pre-hybridize the slides with ready-to-use ***pre-hybridization solution*** for 3-4 hours at **50ºC.**

6 Hybridize the slides ready-to-use ***hybridization solution*** + Probe (Digoxingenin labeled probe: 2-4 ng/ul in hybridization solution) for 12-16 hours at 45ºC.

Note: i)The volume of the pre-hyb,hyb.,andblockingsolutionshouldbejusttheamountthatcancoverthe tissue section part on the slide. ii)Use anImmEdge Pen(purchased from Vector)circle the tissue, then a boundary will be formed, then several drops of solution will be enough to cover the tissue.iii)Duringpre-hybandhyb,inordertoavoidtheevaporation,putwatersoakedpaper towelinaplasticbox,andputanykindofrackonthetopofpapertowel,andputtheslidesonthe rack, cover the plastic box then put the box in an incubator.

C: Washing Steps

1 Wash the slides with 2 x SSC for 10 minutes at 45ºC.

2 Wash the slides with 1.5xSSC for 10 minutes at 45ºC.

3 Wash the slides with 0.2xSSC twice at 37ºC, 20 minutes for each time.

4 Incubate slides with 1 x ***blocking solution*** (dilute 100x blocking solution to 1x with 1x PBS) for 1 hour at room temperature. For some probes, the longer incubation will give the lower background.

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D: Antibody Visualization of Digoxygenin

1 According to the sensitivity of the probe, incubate the slides with 1:100-1:1000 PBS diluted ***AP-conjugated anti-digoxingenin antibody*** for 1-4 hours or overnight at 4ºC

2 Wash the slides with 1xPBS for 3 times at room temperature, 10 minutes each time.

3 Wash the slides with ***1xAlkaline Phosphatase buffer*** twice at room temperature, 5 minutes for each time.

4 According to the abundance of the target RNA, incubate the slides with solution, which is made by adding 6.6 µl NBT and 3.3 µl BCIP into 1 ml 1xAlkaline Phosphatase Buffer, in the dark for 2 to 20 hours at room temperature.

5 Discard the NBT/BCIP solution. Rinse the slides with distilled water

6 Counterstain if required.

Mount the slides.

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