Technical Data Sheet

Technovit® 9100 Short Instructions

#14655

In-situ hybridization for sections

1. Prepare approx. 5µm-thick sections	
2. Section deplastization at room temperature • Xylol • 2-methoxyethyl acetate (2-MEA) • High-purity acetone • Aqua dest. If necesary deplasticize for more time, also without 2.MEA	2 x 20 min 1 x 20 min 2 x 5 min 2 x 5 min
3. Edge sections	
4. Block the endogenous peroxidase 3% H_2O_2 in methanol	30 min
5. Aqua dest.	2 x 5 min
6. Enzymatic digestion • Fast enzyme • Pronase 0,1%, 37°C	10 min bei RT 10 min
7. Aqua dest.	minimum 10 min
8. Fresh aqua dest.	possibly also overnight
9. Let sections dry	
10. Apply probe to the sections, cover with cover glass and seal with Fixogum	
11. Place sections in the hybridizer and hybridize for 2 hours at 55°C	
12. Remove sections from device, remove Fixogum	
13. Place sections in wash buffer	2 x 2 min at RT

Detection:

1. Place AP anti-biotin on the sections	30 min, 37°C
2. Wash in buffer	2 x 2 min, RT

3. Place AP substrate on the sections	30 min, 37°C
4. Wash in buffer	2 x 2 min, RT
5. Place HRP anti dig. on the sections	30 min, 37°C
6. Wash in buffer	2 x 2 min, RT
7. Place HRP substrate on the sections HRP substrate from kit for formalin and plastic-fixated iliac crests: HRP substrate for Shäfer fixated iliac crests: • 10,5 mg of 3-Aminoethylcarbazole (Sigma a 5754) • 1 ml DMSO • in 50 ml acetate buffer pH 5,6 (0,1 molar) • 5µl H_2O_2	30 min, 37°C
8. Rinse sections under running water and if necessary counterstain briefly with diluted hemalaun.	
9. Cover with water	

NOTE:

Ask the corresponding probe manufacturer for additional instructions on ISH.

Reagents

Buffer

2M SODIUM ACETATE STOCK SOLUTION

74,13g of sodium acetate

5,5 ml of glacial acetic acid

ad 500 ml Aqua dest.

0,1M SODIUM ACETATE BUFFER (pH 5,6)

50 ml of 2M sodium acetate stock solution

ad 1000 ml Aqua dest. (adjust pH to 5,6)

1M PHOSPHATE STOCK SOLUTION

112,5g Na_2HPO_4

30g KH_2PO_4

ad 1000 ml Aqua dest.

0,1M PHOSPHATE BUFFER (pH 6,5)

100 ml of 1M phosphate stock solution

ad 1000 ml Aqua dest. (adjust pH to 6,5)

0,01M PHOSPHATE PUFFER (pH 7,4)

10 ml of 1M phosphate stock solution

ad 1000 ml Aqua dest. (adjust pH to 7,4)

0,04M PHOSPHATE BUFFER + 10% SUCROSE (pH 7,4)

40 ml of 1M phosphate stock solution

100g of sucrose

10 ml of 10% NaN_3 solution

ad 1000 ml Aqua dest. (pH 7,4)

1M TRIS STOCK SOLUTION

121,14g of tris

ad 1000 ml Aqua dest.

0,1M TRIS BUFFER (pH 9,4)

100 ml of 1M tris stock solution

ad 1000 ml Aqua dest. (adjust pH to 9,4)

Fixation solutions

Buffered 4% FORMALIN SOLUTION

100 ml of 37% formol

4g of NaH_2PO_4 H_2O

6,5g of Na_2HPO_4

ad 1000 ml Aqua dest. (pH 7,0)

8% PARAFORMALDEHYDE STOCK SOLUTION

40g of paraformaldehyde

ad 500 ml Aqua dest.

1.4% PARAFORMALDEHYDE SOLUTION

35 ml of 8% paraformaldehyde stock solution

65 ml of Aqua dest.

100 ml of 0.04M phosphate buffer +10% sucrose (pH 7,4)

Reaction batches

FAST RED SOLUTION

Put 3 ml of substrate buffer in a plastic tube

Put 1 Fast Red tablet in the solution and dissolve

Add 120 µl of levamisol and mix

Sol. has a shelf life of 1 hour

REACTION SOLUTION: ALKALINE PHOSPHATASE

50 ml 0,1M tris buffer (pH 9,4)

50 mg of Real Blue salt

25 mg of naphthol-AS-BI phosphate (dissolved in 0,5 ml of DMSO/Triton X 100)

REACTION SOLUTION: ACID PHOSPHATASE

50 ml of 0.1M sodium acetate buffer (pH 5,6)

500 µl of hexonium pararosaniline (250 Î¼l 4% pararsonanilin in 2N HCl + 250 µl of 4% sodium nitrate in aqua dest.; vortex for 1 min. let react for 5 min.)

25 mg of naphthol-AS-BI-phosphate (dissolved in 0.5 ml of DMSO/Triton X 100)

REACTION SOLUTION: ASD-CHLOROACETATE ESTERASE

50 ml of 0.1M phosphate buffer (pH 6,5)

15 mg of naphthol AS-D chloroacetate (dissolved in DMSO/TritonX 100)

250 µl of hexonium pararosaniline

Staining solutions

GIEMSA SOLUTION

3% sol., make using the stock solution (Merck)

1-2 drops of 1% acetic acid

LIGHT GREEN

1g light green yellowish

2 ml glacial acetic acid

ad 1000 ml Aqua dest.

PHOSPHOMOLYBDIC ACID / ORANGE G

30 g Phosphomolybdic acid

ad 500 ml Aqua dest.sodium nitrate in aqua dest.; vortex for 1 min; let react for 5 min.)

20 g Orange-G

ad 500 ml Aqua dest.

– Mix both solutions

– Filtrate

PONCEAU ACID MAGENTA AZOPHLOXIN

100 ml Masson sol.

20 ml Azophloxinlsg.

880 ml 0.2% acetic acid

Masson sol.: 1 part sol. A + 2 parts sol. B

Sol. A: 1 g of acid magenta (magenta-S)
ad 100 ml Aqua dest.
– boil
1 ml of glacial acetic acid
– Filtrate

Sol. B: 2 g of Ponceau de Xylidine
ad 200 ml Aqua dest.
– boil
2 ml of glacial acetic acid
– Filtrate

AZOPHLOXIN SOLUTION

0,5 g azophloxin

ad 100 ml Aqua dest.

2 ml glacial acetic acid

Source of Information

Self-experiment with reagents completed by Zytomed Systems GmbH