BioTnA Biospot Kit

Catalog no. TASH01D
Biospot ISH Kit with DAB Brown for DIG labeled probe

Contents

1. Scope of Application

The Biospot kit HRP-DAB is designed to be used for the detection of digoxigenin (DIG)-labeled probes in either formalin-fixed, paraffin-embedded tissue or cell samples by chromogenic in situ hybridization (CISH).

Interpretation of results must be made within the context of the patient's clinical history with respect to further clinical and pathologic data of the patient by a qualified pathologist.

2. Basic Principles

The presence of certain nucleic acid sequences in cells or tissue can be detected with in situ hybridization using labeled oligonucleotide probes. The hybridization results in duplex formation of sequences present in the test object and the specific probe. The Biospot kit HRP-DAB is to be used with any separately available digoxigenin-labeled probe.

Duplex formation of the digoxigenin-labeled probe can be visualized using a primary (unmarked) anti-digoxigenin antibody, which is detected by a secondary, polymerized enzyme-conjugated antibody. The enzymatic reaction of DAB (3,3'-Diaminobenzidine) leads to the formation of strong brown signals that can be visualized by light microscopy at a 10-40x dry lens.

3. Safety Precautions and Disposal

* Read the operating instructions prior to use!
* Do not use the reagents after the expiry date has been reached!
* Avoid any cross-contamination and micro-bacterial contamination of the reagents!
* Some of the system components contain substances (in low concentrations and volumes) that are harmful to health. Avoid any direct contact with the reagents. Take appropriate protective measures (use disposable gloves, protective glasses, and lab garments)!
* If reagents come into contact with skin, rinse skin immediately with copious quantities of water!
* Never pipet solutions with your mouth!
* The disposal of reagents must be carried out in accordance with local regulations!
* A material safety data sheet is available on request for the professional user!

The Biospot Kit HRP-DAB

4.1 Components

The kit is made up of the following components:

Component	100 tests/kit	50 tests/kit	Selected by user
Pretreatment kit			
Heat Pre-treatment Solution,20x	50ml	30ml	
Hydrogen Peroxidase Block	12ml	6ml	
Enzyme Solution	12ml	6ml	
SSC buffer,20x	50ml	30ml	
Hu DNA (+) control probe 5'-DIG	300ul	200ul	◎
Ms DNA (+) control probe 5'-DIG	300ul	200ul	◎
RNA (+) control probe 5'-DIG	300ul	200ul	◎
Detection system			
Hi-Effect Block reagent	12ml	6ml	
Anti-DIG antibody	12ml	6ml	
Polymer HRP labeling	12ml	6ml	
DAB buffer	12ml	6ml	
DAB chromogen,20x	250ul	120ul	
Nuclear Blue Solution	12ml	6ml	

The components of the kit must be stored at 2~8°C. If these storage conditions are followed, the kit will function, without loss of performance, at least until the expiry date printed on the label.

4.3 Test Material

The Biospot kit HRP-DAB has been optimized for the use with formalin-fixed, paraffin-embedded tissue, frozen section and cell samples. When test material is used that has been fixed or embedded in a different manner the test protocol may need to be adapted by the user. Our specialists are available to support you whenever adjustments are necessary.

We recommend the following tissue preparation:

∗ Fixation in 10% neutrally buffered formalin for 24 hours at room temperature.

In order to achieve optimum and uniform fixation and paraffin embedding, the sample size should not exceed 0.5 cm^3.

∗ Standard processing and paraffin embedding

Use premium quality paraffin. Infiltration and embedding should be carried out at temperatures lower than 65°C.

∗ Prepare 5-7µm microtome sections

Draw up the sections onto silane-coated or adhesion slides and incubate at 56°C for 2 hours or 37°C for 16 hours to increase the adhesion.

4.4 Additional Materials

The following reagents, materials, and equipment are not included in the kit:

Reagents and materials

- Digoxigenin-labeled probe
- Adhesive pistol, including hot adhesive, or rubber cement.
- Ethanol 100%, denatured
- Deionized or distilled water
- PBST or TBST buffer
- Xylene

Equipment

- Water bath (boiling, 37-70°C)
- Hot plate
- Hybridization oven (heating oven)
- Staining jars
- Humidity chamber

- Pipet (10µl, 100ul, 1000µl)
- Coverslips (22 mm x 22 mm, 24 mm x 32 mm)
- Light microscope

4.5 Important Information

The following should be kept in mind:

* The tissue and cell sections must not be allowed to dry during the hybridization and staining steps!
* Room temperature is meaning around 25-28°C.
* The temperature for denaturating and washing, described in the protocol, should be used as a guide upon the age and the fixation step of the sample material, an increase or decrease in temperature of the denaturing or wash steps can lead to better hybridization results!

5. The Biospot kit HRP-DAB Protocol

5.1 Preparatory Steps

1. Heat Pre-treatment Solution: Dilute the 20X Heat Pre-treatment Solution in deionized or distilled water and pre-Heat in a covered staining jar standing in a boiling water bath to at least 95°C.

2. Preparing 1X SSC and 2X SSC buffer: Dilute the 20X SSC buffer in deionized or distilled water to 1X and 2X. The diluted SSC buffer should be pre-wormed to 45°C before the post-hybridization step. The diluted SSC buffer could be stored at 2-8°C for 1-2 weeks.

3. TBST Wash Buffer : Prepare one staining jar with 1X TBST Wash Buffer (prepared with deionized or distilled water) and pre-heat to 45°C in a water bath.

4. Enzyme Solution**,** Anti-DIG antibody**,** Polymer HRP labeling, Nuclear Blue Solution: Bring reagents to room temperature before use.

5. Preparation of DAB Solution: Prior to using, dilute the DAB chromogen (20X) in DAB buffer and mix well. The solution is stable for 4 hours at room temperature.

5.2 Pretreatment (Dewax/Proteolysis)

1. Incubate slides for 15 min at 56-60°C (e.g. on a hot plate)

2. Dewax in xylene for 8-10 minutes for three time.

3. Rehydration in gradient alcohol (100% x2, 90%x1, 80%x1,70%x1) for four minutes pre concentration. Keeping the slides in PBST wash buffer before staining.

4. De-peroxidase in Hydrogen Peroxidase Block for 10 minutes.

5. Wash slides 2 times for 1 minute in PBST wash buffer.

6. Pre-heat pre-treatment Solution in a covered standing in a boiling water bath to at least 95°C

7. Place slides into the pre-heated Heat Pre-treatment Solution and incubate for 30 minutes.

8. Cooling down at least 15 minutes and immerse slides in deionized or distilled water and drain off or blot off the water.

9. Apply (dropwise) Enzyme Solution to the tissue/cell section and incubate for 10 minutes at 37°C in a humidity chamber. Depending on multiple factors, e.g. nature and duration of fixing, thickness of sections, and nature of tissue/cells, different incubation times may be required. Generally, we recommend ascertaining the optimum time for proteolysis in pre-tests.

10. Rinse slide in DD water for 1 minutes.

11. Dehydration: in 70%, 85%, 95%, and 2x 100% ethanol, each for 2 min

12. Air dry.

5.3 Denaturation and Hybridization

1. Preparing the probe and diluted in DEPC water to 1uM (suggest initial test). Mixing probe well and pipette enough probe each onto individual samples distribute dropwise on the whole target area.

2. Avoiding trapped bubbles, cover the samples with a coverslip (22 mm x 22 mm) or larger. Seal the coverslip, e.g. with a layer of hot glue from an adhesive pistol or with rubber cement.

3. Denature the slides at 96°C for 10 min, e.g. on a hot plate

4. Transfer the slides to a humidity chamber and hybridize (e.g. in a hybridization oven) for 2-16 hours at 37°C or 55°C. It should be tested by user.
The sections dried will cause false outcome during the hybridization step.

5.4 Post-Hybridization and Detection

1. Carefully remove the rubber cement or glue.

2. Remove the coverslip by submerging in 1X PBST Wash Buffer.

3. Immerse slides in 45°C pre-wormed 2X SSC and 1X SSC buffer sequentially for 10

minutes to wash unbinding probes.

4. Wash 5 minutes in 1X PBST wash buffer.

5. Apply Hi-Effect Block reagent dropwise (3-4 drops per slide) to the sections and incubate for 30 to 60 minutes at room temperature in a humidity chamber to reduce non-specific background staining.

6. Wash 5 minutes in 1X PBST wash buffer.

7. Apply Anti-DIG antibody dropwise (3-4 drops per slide) to the sections and incubate for 30 min at 37°C or 1 hour at room temperature in a humidity chamber

8. Wash slides in 1X PBST wash buffer 1 minute for three times.

9. Apply Polymer HRP labeling dropwise (3-4 drops per slide) to the sections and incubate for 30 minutes at room temperature in a humidity chamber.

10. Wash slides in 1X PBST wash buffer 1 minute for three times.

11. During the wash steps, prepare DAB working solution. Dilute the DAB chromogen (20X) in 19 times volume of DAB buffer.

12. Apply DAB working solution dropwise (3-4 drops per slide) to the sections and observe in microscope during the color development. Terminate the color development by user in PBST wash buffer to remove the DAB solution.

13. Transfer slides into a staining jar and wash 2 minutes in running tap water.

14. Counterstain with Nuclear Blue Solution for 1-3 minutes**.**

The counterstain depends on the nature of tissue/cell used and should be optimized. Darker counterstain will reduce the contrast and obscure positive staining signals.

15. Transfer slides into a staining jar and wash 2 min in running tap water.

16. Dehydration in 70%, 85%, 95%, and 100% ethanol, each for 2 minutes. Air dry and cover sections in coverslips by mounting medium.

17. Observe in light microscopy.

STORAGE & STABILITY:

Store at 2-8°C. Do not freeze. The user must validate any other storage conditions. The reagents must be returned to the storage conditions identified above immediately after use. When properly stored, the reagent is stable to the date indicated on the label. Do not use the reagent beyond the expiration date. There are no definitive signs to indicate instability of this product; therefore, positive and negative controls should be tested simultaneously with unknown specimens. If unexpected results are observed which cannot be explained by variations in laboratory procedures and a problem with the reagent is suspected, contact Technical Support at info@biotna.net or your distributor service.