NOVUS BIOLOGICALS
a bio-techne brand

IMPORTANT

Before You Begin

Read through the protocols. Protocols should serve as starting points for your experiements. As always, you should optimize protocols for your purposes.
Novus Tissue Array Slides are quality controlled by immunohistochemistry. The suitability of the various donor tissue cores on slides for other application such as DNA isolation, FISH, and *In Situ* hybridization should be empirically determined by the researcher.

1 Standard Protocol for Immunohistochemistry

1. **Deparaffinization and Hydration**
   a. Incubate slides in a dry over at 62°C for 1 hr. Slides should be maintained in a vertical orientation to allow complete removal of the paraffin.
   b. Dewax slides in xylene for 5 x 4 min.
   c. Hydrate slides in 100%, 95% and 75% ethanol for 2 x 3 min each.
   d. Immerse slides in tap water for 5 min.
   Note: Please make sure to perform the deparaffinization and Hydration Steps prior to performing antibody staining or antigen retrieval (except for antigen retrieval Method 4). This will ensure complete deparaffinization of the Tissue Array Slides.
2. Perform an antigen retrieval method (optional).
3. Quench endogenous peroxidase (optional).
   a. Immerse slides in 3% hydrogen peroxide solution for 6 min.
   b. Wash slides in PBS for 3 x 5 min.
4. Primary antibody labeling
   a. Incubate slides with blocking serum for 30 min.*
   b. Blot excess serum from sections.
   c. Incubate with diluted primary antibody**
   d. Wash slides in PBS for 3 x 5 min.
5. Secondary antibody labeling
   a. Incubate slides with biotin-conjugated secondary antibody for 30 min.*
   b. Wash slides in PBS for 3 x 5 min.
6. Detection
   a. Avidin-biotin-peroxidase complex method.
      i. incubate slides with Avidin-Biotin Complex for 30 min.*
      ii. Wash slides in PBS for 3 x 5 min.
   b. Chromogenic reaction
      i. Incubate slides in fresh DAB solution for 2 min.
      ii. Stop the reaction by washing in tap water.
7. Counterstain in Meyer's hematoxylin for 10 sec.
8. Dehydrate slides in 75%, 80%, 95% and 100% ethanol.
9. Clear slides in xylene 4 X 5 min.
10. Mount cover slide with Permount.

*Blocking serum, secondary antibody and avidin-biotin complex are included in most commercially available immunostaining kits. We have used the ABC Kit from Vector Lab (Vecastain Elite ABC Kit), Cat no. PK-6102 for mouse antibodies and Cat no. PK-6101 for rabbit antibodies.

**Incubation times varies with antibodies. In the first trial when the optimal time is unknown, incubate monoclonal antibodies for 2 hr at RT or overnight at 4°C and incubate polyclonal antibodies for 1-1.5 hr at RT.

***We use DAB/Ni substrate kit from Vector Labs, Cat no. SK-4100.

2 Standard Protocol for Antigen Retrieval

The following four methods are suggestions only. Other protocols can be used on the array slides.
The Deparaffinization and Hydration steps in the Immunohistochemistry Protocol must be performed prior to using Antigen Retrieval Methods 1, 2 or 3.

Method 1 (Microwave Technique)

1. Immerse slides into citrate buffer (0.01 M, pH6.0) or EDTA buffer (1mM, pH8.0).
2. Microwave (700 W or high) for 5 min, add buffer if necessary.
3. Microwave (medium) for 5 min, add buffer if necessary.
4. Microwave (low) for 5 min.
5. Immerse in cold PBS (pH7.6) for 3 x 5 min.

Method 2 (Autoclave Technique)

1. Immerse slides in citrate buffer (0.01 M, pH6.0) or EDTA buffer (1mM, pH8.0).
2. Autoclave for 2 min at 120°C.
3. Cool slide to RT.
4. Wash slides in PBS for 3 x 5 min.

Method 3 (Enzyme Treatment Technique)

1. Incubate slides with one of the following enzyme treatments, adjusting the incubation time according to the needs of your antibody:
    a. Pronase: 0.05% (w/v) in PBS at 37°C.
    b. Trypsin: 0.05% (v/v) in PBS at 37°C.
    c. Pepsin: 0.05% (v/v) in 2N HCL at 37°C.
    d. Proteinase K (0.1% in TBS) at RT.
2. Wash slides in PBS for 3 x 5 min.

Method 4 (Decloaking Technique)

1. Dry slides at 60°C for 1 hr (deparaffinization is not necessary).
2. Immerse slides in BORG Decloaker solution (BIOCARE: www.biocare.net).
3. Autoclave in Decloaking Chamber (BIOCARE).
4. Cool slides to RT.
5. Wash slides in PBS for 3 x 5 min.

3 Standard Protocol for DNA Isolation

1. Deparaffinization

a. Incubate tissue array slides in a dry oven at 60°C for 1 hr. Slides should be maintained in a vertical orientation to allow complete removal of the parafin.
b. Dewax slides in Xylene for 5 x 4 min.
c. Remove Xylene in 100% ethanol x 3 changes.
d. Air dry slides.

2. Tissue Collection

a. Wet the tissue with 20% glycerol buffer (5 ul for a single core).
    - Glycerol buffer: 20% glycerol in 1X TE (pH8.0).
b. Scratch the tissue with a 26G syringe needle.
c. Collect the cores from 2-10 slides into 1.5 ml eppendorf tube (use laser capture microdissection if necessary).
d. Add 200 ul DNA extraction buffer.
    - DNA extraction buffer: 100 mM NaCl, 10 mM Tris, 25 mM EDTA, 0.5% SDS (pH8.0).
e. Add 5 ul of 20 mg/ml protein kinase solution.
f. Incubate at 55°C overnight with shaking.

3. Extraction

a. Add equal volume of phenol/chloroform/isoamyl alcohol (~25:24:1).
b. Vortex for 5 min.
c. Centrifuge at 14,000 rpm for 10 min.
d. Transfer aqueous layer into a new tube (~150 ul).

4. Precipitation

a. Add half volume of 7.5 M ammonium acetate (~75 ul).
b. Add 2.5 volume of 100% cold ethanol (~560 ul).
c. Incubate at -20°C for 30 min.
d. Centrifuge at 14,000 rpm at 0°C for 10 min.
e. Wash with 500 ul of 70% ethanol, centrifuge again.
f. Dry at RT.
g. Dissolve the DNA pellet in distilled water (pH 8.0).

** We usually collect the cores of 2 to 10 slides, and get 200 ng DNA.*

4 Standard Protocol for FISH

1. **Deparaffinization**
   a. Incubate slides in a dry over at 62°C for 1 hr. Slides should be maintained in a vertical orientation to allow complete removal of the paraffin.
   b. Dewax the slides in xylene for 10 x 3 min
   c. Hydrate slides in 100% ethanol 5 x 2 min.
   d. Air dry.

2. **Slide pretreatment**
   a. Use a commercial Paraffin Pretreatment Kit designed to treat the tissues prior to FISH and follow the manufacturer's instructions.

3. **Hybridization**
   a. Prewarm the slides to 45° on a slide warmer.
   b. Add probe solution to the slides.
   c. Place a cover glass on the probe and seal with rubber cement.
   d. Incubate at 42° for 4-16 hr.

4. **Wash**
   a. Remove rubber cement.
   b. Immerse slides in 2X SCC/50% formamide for 15 min in a 45°C water bath.
   c. Allow the cover glass to drop off smoothly.
   d. Wash slides in 2X SCC/50% formamide for 2 x 10 min in a 45°C water bath.
   e. Wash slides in 2X SCC/50% formamide for 10 min at RT.
   f. Wash slides in 2X SCC/0.01% NP-40 for 5 min.

5 Standard Protocol for *In Situ* Hybridization

The following instruction is provided as a guideline for using digoxigenin-labeled probe. Other labeled probes or protocols may be used with tissue array slides.

Part I. The design and generation of probes with digoxigenin

1. Template preparation
   a. For cDNA insert in transcription vector including promoter for SP6, T7, or T3 RNA polymerase: linearize cDNA with restriction enzyme, and purify linearized cDNA by ethanol precipitation.
   b. For PCR-generated fragments:
      - Prepare primers, such as SP6, T7, or T3 promoter sequence.
      - Generate PCR fragments using PCR reaction.
      - Purify PCR fragments using a commercially available kit.

2. Probe construction
   a. Prepare cRNA probe reaction mixture as follows:
      - Template (1- 2 ug), 1 ul
      - Appropriate RNA polymerase, 2 ul
      - 10X transcription buffer, 2 ul
      - 10X DIG RNA labeling mix, 2 ul
      - Sterile RNase free water, 13 ul

      Total volume 20 ul

3. Incubate reaction mixture for 2 hr at 37°C, add 2 ul DNase I.

4. Incubate for 15 min at 37°C, add 2 ul 0.2 M EDTA.

Part II. *In Situ* Hybridization

1. Deparaffinization and Hydration
   a. Incubate in a dry oven at 62°C for 1 hr. Slides should be maintained in a vertical orientation to allow complete removal of the paraffin.
   b. Dewax slides in xylene for 10 x 3 min.
   c. Hydrate slides in 100%, 95%, and 75% ethanol for 5 x 2 min each.
   d. Immerse slides in DEPC-treated PBS for 5 min.

2. Post-fixation
   a. Immerse slides into 4% buffered paraformaldehyde for 10 min.

3. Proteinase digestion
   a. Immerse slides in 10 ug/ml proteinase K solution for 20 min at RT.

4. Quenching of endogenous alkaline phosphatase
   a. Immerse slides in 0.2N HCl for 1 hr.
   b. Wash slides in DEPC-PBS for 5 min.

5. Electrostatic interaction inhibition
   a. Immerse slides in 0.1 M TEA for 10 min.
   b. Immerse slides in 0.1 M TEA including 0.25% acetic acid for 10 min.
   c. Wash slides in DEPC-PBS for 5 x 2 min.
   d. Dry slides on air.

6. Hybridization
   a. Prewarm hybrisol for 20 min at 50°C prior to mixing probe. Mix DIG-labeled probe (2 ug) with 100 ul prewarmed hybrisol.
   b. Incubate slides with probe for 16 hr at 50°C in a HYBrite™ instrument.

7. Washing
   a. Immerse slides in 2X SSC/50% formamide for 15 min in a 50°C water bath.
   b. Remove coverslip from slides.
   c. Wash slides in 2X SSC/50% formamide for 2 x 30 min in a 50°C water bath.
   d. Wash slides in 2X SSC for 20 min at RT.
   e. Wash slides in washing buffer (or DIG1 solution) for 5 min at RT.

8. Incubate slides with blocking solution (or DIG2 solution) for 2 hr.

9. Blot excess blocking solution from slides, and incubate with anti-DIG antibody for 2 hr at RT or overnight at 4°C.

10. Wash slides in washing buffer (or DIG1 solution) including Tween-20 for 4 x 30 min on a shaker.

11. Detection
    a. Incubate slides in NBT/BCIP solution with 2 M MgCl2 and 1 mM levamisole or 4.5 ul NBT and 3.5 ul BCIP solution in 1 ml DIG3 solution with 25 ul MgCl2 and 1 mM levamisole.
    b. Stop the reaction by washing slides in DIG4 solution for 10 min.
12. If needed, counterstain with methyl green.
13. Mount the slides with glycerol gels.