

PANTOMICS MINI IHC KIT (IHC-PRM05) & PROTOCOL

1.0 Materials and Reagents Provided

Table 1 - Pantomics Mini IHC Kit (IHC-PRM05)

Reagent	Form	Volume
Peroxidase blocking solution (H ₂ O ₂)	12X	2 ml
Antigen retrieval solution (pH 9.5)	10X	50 ml
Pre-blocking solution (antibody diluent)	ready-to-use	7 ml
Post-antibody blocking solution	ready-to-use	7 ml
Secondary poly-HRP-anti-Rabbit/mouse Ig	ready-to-use	5 ml
DAB A	30X	1 ml
DAB B	30X	1 ml

Note: Please store all the above reagents at 4°C.

1.1 Notes on DAB substrate solution Preparation

- Add one drop of DAB A + one drop of DAB B to 1ml distilled water
- Use immediately

2.0 Materials and Reagents Required

- Xylene
- Ethanol (100%, 95%, 70%)
- TBST washing buffer: Tris Base Saline buffer with 0.1% Tween-20 (50 mM Tris, pH 7.6, 150 mM NaCl, 0.1% Tween-20)
- Distilled water (dH₂O)
- Mayer's Hematoxylin QS
- Permanent Mounting medium
- Rabbit or mouse primary antibody

2.1 Equipment required

- Slide Rack
- Heat resistant slide jar or dish
- Water bath, rice cooker or pressure cooker
- Pipettors and pipette tips of various sizes
- Glass coverslips

3.0 Procedure

Please read through entire procedure before beginning.

3.1. Deparaffinization/rehydration

- Bake slides in an oven at 60 °C for 30mins to 1 hour.



- b. De-paraffinize and hydrate tissue sections by soaking the slides in:
 - i. Xylene 2 X 5 minutes
 - ii. 100% ethanol, 2 X 5 minutes
 - iii. 95% ethanol, 1 X 3 minutes
 - iv. 70% ethanol, 1 X 3 minutes
 - vi. Distilled water, 1 X 3 minutes

3.2. Peroxidase inactivation

- a. Add 2 drops of the peroxidase blocking solution into each ml of dH₂O.
- b. Cover the tissue sections with the above solution and incubate the slides at R/T for 10 min.

3.3. Antigen retrieval with heat

Dilute the 10X retrieval with dH₂O. The diluted antigen retrieval buffer can be saved for repeated use (at least for a few times).

Water bath or rice cooker method (recommended):

- a. Pre-heat the bath or cooker with heat-resistant jar or box filled with retrieval Buffer to >95°C (do not let the buffer boil actively).
- b. Immerse the slides in the buffer and cover the jar with the lid or plastic clean film.
- c. Continue to heat or "cook" the slides at >95°C for 5-8 minutes.
- d. When water bath or rice cooker is turned off, unplug the cooker and remove the jar to the bench top.
- e. Allow it to cool down for 20 min.

Pressure cooker method (need more retrieval buffer):

- a. Heat the antigen retrieval buffer to boiling by using a stainless steel pressure cooker. Do not lock lid at this moment.
- b. When the buffer boils, power off, put a heat-resistant rack with tissue array slides in the cooker and make sure that the buffer covers the slides.
- c. Lock the lid, cook the array slides for 5 minutes with moderate power.
- d. Remove the pressure cooker and cool it down under tap water. When the indicator sinks, open the lid and remove the jar or box and let it cool at room temperature for 8 minutes. Transfer the slides from the jar or box to tap water and rinse them for 3 minutes.

Microwave oven method:

- a. Heat the antigen retrieval buffer in a heat-resistant jar to boiling, then put tissue array slides in the buffer. Cover the jar with lid or plastic clean film.

- b. Set the power to simmer or 1/3 of energy level in the microwave oven for 10-15 min. **Do NOT** let the buffer boil.
- c. Remove the jar and leave it at R/T to cool for 15 minutes.

3.4. IHC Detection

- a. Rinse the slides with 2 changes of TBST, 2 minutes each.
- b. Block the slides with the Pre-blocking solution for 10 mins, then tip off the solution without rinsing or washing.
- c. Cover the tissue array sections with primary antibody diluted in the Pre-blocking solution or TBST (100-200µl per section)
- d. Incubate the array sections with the antibody at R/T for 30 to 1 hour in a humidified chamber.
- e. Wash slides three times with TBST (3 min each).
- f. Incubate the sections with Post-blocking for 20 mins.
- g. Wash slides twice with TBST (3 min each).
- h. Incubate the sections with the poly-HRP-anti-Rabbit/mouse Ig at R/T for 30 to 50 mins (100-200µl per section).
- i. Wash the slides three times with TBST (3 min each).
- j. Incubate the sections with the fresh-made DAB substrate solution at R/T until suitable staining develops (generally 2–5 min).
- k. Rinse the slides with running tap water.
- l. Counterstain the sections with Mayer's Hematoxylin (30 to 60 seconds).
- m. Rinse the slides well with running tap water.
- n. Dehydrate the sections through 95% ethanol for 2X2 minutes, 100% ethanol for 2x3min. Clear them in xylene for 2x3min.
- o. Mount the array sections with coverslips of appropriate size using Permount medium.

4.0 Troubleshooting

Table 2 – TroubleShooting

Problem	Possible cause
No staining	<ul style="list-style-type: none"> - Steps of the protocol or reagents not followed in proper order - Antigens are destroyed - Tissue is incorrectly fixed/ or processed
Weak Staining	<ul style="list-style-type: none"> - Incomplete deparaffinization (with high background) - Inadequate epitope retrieval - Inadequate reagents incubation time - Inappropriate tissue fixation method used - Incorrect substrates preparation
High Background	<ul style="list-style-type: none"> - Incomplete deparaffinization - Incomplete blocking of peroxidase activity - Slides not thoroughly washed - Inappropriate tissue fixation method used



Pantomics, Inc

Phone: 415-863-2380

Support: info@pantomics.com

Web: www.pantomics.com

Advancing Biomedical Science Through Tissue Arrays

- | | |
|--|---|
| | <ul style="list-style-type: none">- Sections dried during staining procedure- Excessive incubation with substrates |
|--|---|