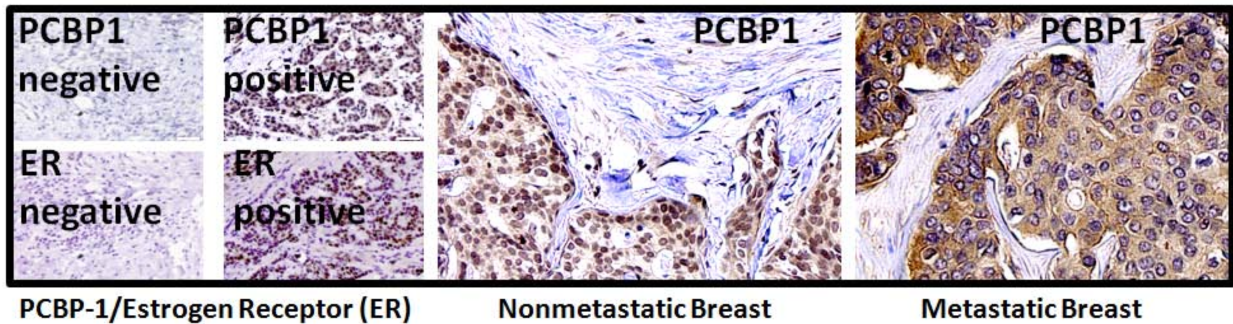
Alper Biotech PCBP-1 IHC Kit

For immunohistochemical staining of PCBP1 (Poly(rC)-binding protein 1 in FFPE Tissue

Cat. No. AB1



PCBP-1 IHC Kit is a sensitive immunohistochemistry kit that is specific for the detection of PCBP-1 protein in formalin fixed, paraffin-embedded (FFPE) tissue sections. Alper **Anti-PCBP-1 monoclonal antibody recognizes the native form of PCBP-1*.***

Image *shows immunohistochemical staining of paraffin-embedded human breast adenocarcinoma tissue sections stained with Alper-PCBP-1 monoclonal antibody using the PCBP-1 IHC Kit (Cat No. AB1-Lot 01) at 20x magnification.*

Intended Use:

For Research Use Only.

Introduction

http://www.genecards.org/cgi-bin/carddisp?gene=PCBP1

Reagents provided in the kit

The materials listed are sufficient for 20 tests. The number of tests is based on the use of 200 μL each of ready-to-use reagent per slide.

- **Retrieval Buffer (10X)**
  30mL
  Dilute at 1:10 using distilled or deionized water prior to staining; unused working solution may be stored at room temperature (20-25 ^{0}C).
- **Wash Buffer (10x)**
  30mL, Tris buffered saline with Tween 20 (pH7.6)
  Dilute at 1:10 using distilled or deionized water prior to staining; unused working solution may be stored at room temperature.
- **Peroxidase Blocking Buffer**
  5mL, 3% Hydrogen Peroxide
  Ready-to-use
- **Blocking Reagent**,
  4mL, Background Sniper - Biocare Medical; Cat No. BS966 G
  Ready-to-use
- **Human PCBP-1 Monoclonal Mouse IgG1 antibody**
  500μg/mL; 100μl total (50μg)
  Dilute in Antibody Diluents immediately before use (recommend use at 1:50-1:500 dilution).

- **Antibody Diluents**
  5mL
  Ready-to-use
- **MACH3 Mouse Probe**
  4mL, Biocare Medical; Cat No. M3M530
  Ready-to-use
- **MACH3 Mouse HRP Polymer**
  4mL, Biocare Medical; Cat No. M3M530
  Ready-to-use
- **DAB Chromogen**
  0.2mL, Diaminobenzedinetetrahydrochloride (DAB) substrate solution
  Before use, add 20 μL DAB substrate solution to 1 mL of substrate buffer. The prepared Substrate working solution should be stored at 2-8 °C and used within 5 days.
  Do not expose DAB components to direct or bright light during storage and staining process.
- **DAB Substrate Buffer**
  5mL
  Ready-to-use

Materials required but not included in the kit

Reagents:

- Xylene
- Ethanol
- Hematoxylin
- Permanent mounting media
- Distilled or deionized water

Lab Equipment:

- Steamer or microwave oven or domestic steel pressure cooker (for antigen retrieval)
- General lab equipment for immunohistostaining such as slide racks, staining jars, forceps, cover slips, timer, pipettes, etc.
- Microscope equipment and accessories

Storage and stability

Store PCBP-1 IHC Kit at 2-8 °C. The kit is stable for one year at 4 °C.
For long term storage, aliquot the antibody into small volumes and store at -20^{0}C.
Do not use after expiration date.

Precautions

Take reasonable precautions when handling reagents. Use disposable gloves when
handling suspected carcinogens or toxic materials (examples: DAB, xylene). Unused solution should be disposed according to applicable local, state and federal regulations.

Staining Protocol

The PCBP1 Immunohistostaining Kit has been designed for the staining of tissues that have been fixed (usually in neutral buffered formalin) and subsequently embedded in paraffin before sectioning. *The protocol written here has been optimized for specific PCBP-1 protein staining and was developed using a breast cancer tissue microarray and process guidelines provided by The Yale Pathology / Yale Cancer Center Tissue Microarray Facility.*

This protocol is recommended as a starting point. Whenever using a new antibody or immunohistochemistry kit, optimization by the individual end-user may be required.

Note:

- All reagents should be allowed to equilibrate to room temperature (20-25 ^{0}C) before use, and the whole staining process should be performed at room temperature except for the steps specifically described below**.**
- Do not allow specimens to dry during the staining procedure. Specimen drying may cause increased non-specific staining and background.
- Some tissue arrays may need to bake to remove over-covered paraffin prior to the procedure. Check tissue array manufacturer's instruction.

Deparaffinization and rehydration

Prior to staining, tissue sections must be deparaffinized and rehydrated. Incomplete removal of paraffin can cause poor staining of the section.

Step 1. Immerse slide in xylene and incubate for 15 minutes. Repeat once with fresh xylene for another 15 minutes.
Step 2. Immerse slide in xylene: ethanol (1:1) for 5 minutes.
Step 3. Immerse slide in 100% ethanol for 5 minutes, and follow with immersion in 95%, 75% and 50% ethanol for 3 minutes each.
Step 4. Rinse slide with reagent-quality water for 5 minutes; keep in water until ready to perform antigen retrieval.

Heat induced antigen retrieval (HIAR)

Most formalin-fixed tissue requires an antigen retrieval step before immunohistochemical staining can proceed. Heat induced antigen retrieval can be performed using a steamer, pressure cooker, or a microwave. The retrieval time written in this protocol is based on using a retrieval steamer. The heating time may need to be adjusted if you use a different device and method.

Step 1. Fill plastic Coplin jar/container with Retrieval Buffer.

Step 2. Place the Coplin jar/container in steamer.

Step 3. Turn on steamer and preheat to 90-100 °C. Carefully put slide into the Coplin jar/container and steam for 20 min (95-100 °C)

Step 4. Turn off the steamer, remove the Coplin jar, place at room temperature and allow slide to cool for 20 min.

Step 5. Rinse slide by incubation of slide in wash buffer for 3 minutes. Repeat this step twice and begin staining procedure.

Staining procedure

Step 1. Tap off excess washing buffer. Apply enough Peroxidase Blocking Buffer to cover specimen, and incubate for 5 minutes.

Step 2. Rinse slide by incubation of slide in wash buffer for 3 minutes. Repeat this step twice with fresh wash buffer.

Step 3. Tap off excess washing buffer. Apply enough Blocking Reagent to cover specimen and incubate for 5 minutes.

Step 4. Rinse slide by incubation of slide in wash buffer for 3 minutes. Repeat this step twice with fresh wash buffer.

Step 5. Tap off excess washing buffer. Apply enough anti-PCBP-1 antibody (recommend 1:50-500 dilution in antibody diluents) to cover specimen, and incubate for 1 hour.

Step 6. Rinse slide by incubation of slide in wash buffer for 3 minutes. Repeat this step twice with fresh wash buffer.

Step 7. Tap off excess washing buffer. Apply enough Mach3 probe to cover specimen, and incubate for 15 minutes.

Step 8. Rinse slide by incubation of slide in wash buffer for 3 minutes. Repeat this step twice with fresh wash buffer.

Step 9. Tap off excess washing buffer. Apply enough Mach3 polymers to cover specimen, and incubate for 15 minutes

Step 10.Rinse slide by incubation of slide in wash buffer for 3 minutes. Repeat this step twice with fresh wash buffer.

Step 11.Tap off excess washing buffer. Apply enough DAB substrate solution to cover specimen and incubate until desired stain intensity develops.

Step 12. Rinse slide in tap water for 3 minutes.

Step 13. If desired, complete counterstain (See instruction for hematoxylin counterstaining). Rinse to clear.

Step 14. Immerse slide in 70%, 80%, 95%, 100% ethanol for 2 minutes each, and follow in xylene for 2 minutes twice.

Step 15. Dry and mount slide.

Instruction for Hematoxylin counterstain

Step 1. Immerse slide in hematoxylin solution. Incubate for 30 seconds to 5 minutes, depending on the strength of hematoxylin used.

Step 2. Rinse to clear with tap water and continue by dehydration from Step 14.

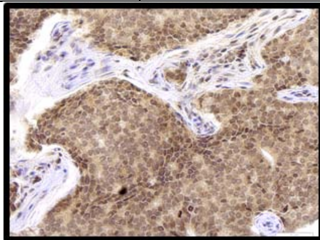
Troubleshooting

Problems	Possible Causes	Solutions
Weak or no staining	1. The primary antibody concentration is too low.	The concentration of the primary antibody can be increased from 1:500 up to 1:50 depending on the tissue section source.
	2. Incomplete removal of paraffin	Deparaffinize sections longer or change to fresh xylene; some tissue array may need to bake to remove over-covered paraffin.
	3. Tissues over-fixation	Increasing the concentration of primary antibody to 1:40; if this does not work, reduce duration of post-fixation.
	4. Not efficient antigen retrieval	Adjust antigen retrieval time based on the situation of section fixation and retrieval device you used.
	5. Reagents not used in proper order or omitted steps	Review notes and procedure used.
	6. Expired antibody or reagents	Check kit expiration dates and kit storage condition

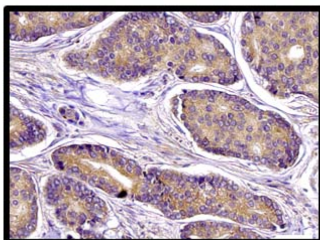
Over staining	1. Too long incubation time of primary antibody, or too high temperature when doing staining 2. Too long incubation time of DAB substrate. 3. Slide dried during staining process	Depending on tissue sections, the incubation time of primary antibody can be reduced to 30 minutes; Check the room temperature range is at 20-25 ºC when doing staining. Reduce incubation time of DAB substrate Avoid sections to dry during staining process.
High background	1. Incomplete removal of paraffin 2 Sections dried during staining process 3 Slide not rinse thoroughly 4 Antigen over-retrieval	Deparaffinize sections longer or change fresh xylene. Do not allow sections to dry during staining process; use humid container during incubation of primary antibody. Use fresh solution in buffer jars; rinse at least three times between steps. Optimize antigen retrieval time if you used microwave or pressure cooker for retrieval.

Note: While the protocol described above has been optimized for immunohistochemical uses, Alper anti-PCBP-1 monoclonal antibody has been found suitable for other purposes as well, such as Indirect-Immunofluorescence Staining, Western Blotting, Immunoprecipitation, Fluorescence-Activated Cell Sorting or ELISA.

Weak or no staining	7. Incomplete removal of paraffin 8. Tissues over-fixation 9. Not efficient antigen retrieval 10. Reagents not used in proper order or omitted steps 11. Expired antibody or reagents	Deparaffinize sections longer or change to fresh xylene; some tissue array may need to bake to remove over-covered paraffin. Increasing the concentration of primary antibody to 1:40; if this does not work, reduce duration of post-fixation. Adjust antigen retrieval time based on the situation of section fixation and retrieval device you used. Review notes and procedure used. Check kit expiration dates and kit storage condition



Endometrial Cancer 20X



Prostate Cancer 20X