ab288086 – Ready-to-use IHC Kit

For use in IHC/ICC staining of membrane, cytoplasmic and nuclear antigens.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab288086

Storage and Stability

Entire kit can be stored at 4°C. Before using the kit, bring reagents to RT.

Materials Supplied

Item	Quantity	Storage Condition
Primary antibody dilution buffer	5 mL	4°C
HRP-anti mouse/rat/rabbit polymer	5 mL	4°C
Peroxidase Bloc	7.5 mL	4°C
Protein Blocking Solution	5 mL	4°C
Reagent BS (buffer & substrate)	5 mL	4°C
Reagent C (conc. DAB chromogen)	1 mL	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Washing buffer
- Antigen retrieval reagents and equipment
- Positive or negative control
- Primary Antibody
- Counterstain
- Clearing agents (xylene and graded alcohols)
- Glass slides and coverslips
- Mounting medium

IHC/ICC procedure for frozen, paraffin sections and cell smears

- Bring all reagents and samples to room temperature prior to the assay.

1) Deparaffinize and hydrate tissue sections (mounted on glass slides) through xylene or other clearing agents and graded alcohols.
   Δ Note: For frozen sections or cell smears; use unfixed, acetone fixed or appropriate fixative for the antigen in question. For cell smears it may be necessary to permeabilize the cells by detergent - Triton or NP40 (0.1-0.2% in PBS for 10 min.). Tween20, Saponin, Digitonin or leucoperm (0.2-0.5% in PBS for 10-30 min.) are suitable for cytoplasmic or plasma membrane antigens.
2) Wash 2-3 times with distilled or deionized water.
3) Incubate sections/cell smear with Peroxidase Block (clear cap) for 5-10 minutes at room temp. Wash with distilled water 3X.
   Δ Note: If antigen retriever (Trypsin AR-6541, Pronase AR-6542, Pepsin AR-6543, Citrate buffer AR-6544, Buffer w EDTA pH 8.5 AR-6545, Tris buffer pH 10 AR-6546) is required, it can be applied at this step.
4) Wash slide with PBS or Tris saline buffer (with 0.02-0.05% non-ionic detergent, Triton X100, Tween 20 or NP-40) 3X.
5) Incubate sections/ cell smear in Protein blocking solution (blue cap), for 5-10 minutes at RT.
6) Incubate sections/cell smear with primary antibody (not supplied, only buffer is supplied for dilution) for 20-30 minutes at RT. The primary antibody dilution buffer supplied can also be used as a negative control.
7) Wash slide with PBS 5-7X. Incubate with One-Step HRP polymer (brown cap) for 20-30 minutes at RT.
8) Wash slide 5-7 times with PBS or TBS preferably with 0.025-0.05% non-ionic detergent (Triton x-100 or Tween- 20 or NP-40) without sodium azide.

Δ Caution: Peroxidase reagents are destroyed by sodium azide and should be avoided in all buffers and regents.

9) Wash slide with deionized or distilled for 2-3X.
10) In a test tube, add 1 ml of Reagent BS & 50 µl of Reagent C (DAB chromogen). Mix well. (This ready-to-use DAB reagent is good for 7- 8 hr.)
11) Add few drops of ready to use DAB reagent on tissue slides & wait for 6-10 minutes at RT. (Higher temp. 37°C can also be used for DAB chromogen, however the incubation time should be determined by the individual lab)
12) Wash 5-7X with PBS, followed by rinsing with distilled or deionized water.
13) Incubate with counterstain compatible with DAB (Not supplied) for 30-60 sec. We prefer haematoxylin.
14) Wash slide with distilled/deionized water.
15) Clear tissue slides by placing into graded alcohols and finally xylene
16) Mount with appropriate mounting medium.

Δ Note: These are guidelines, the optimum incubation times for these reagents and reactions should be determined by the individual user.

Technical Support

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