abcam

Product datasheet

Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free ab217173

KO VALIDATED Recombinant RabMAb

2 Abreviews 16 References 20 Images

Overview

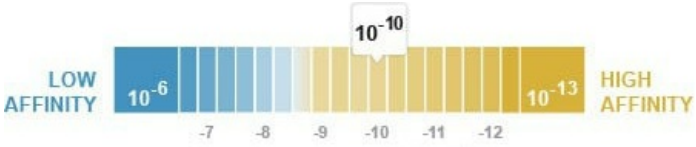
Product name	Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free
Description	Rabbit monoclonal [EP1628Y] to Cytokeratin 8 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, IP, IHC-P, ICC/IF, Flow Cyt (Intra), IHC-Fr
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human Cytokeratin 8 aa 300-400 (C terminal). The exact sequence is proprietary. Database link: **P05787**
Positive control	WB: A431, HeLa and MCF7 cell lysates. A431 cell lysate, HeLa cells or human breast adenocarcinoma tissue. Flow cyto (intra): NIH3T3 cells
General notes	ab217173 is the carrier-free version of **ab53280**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Dissociation constant (K_D)	$K_D = 4.60 \times 10^{-10}$ M



Learn more about K_D

Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1628Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab217173 in the following tested applications.

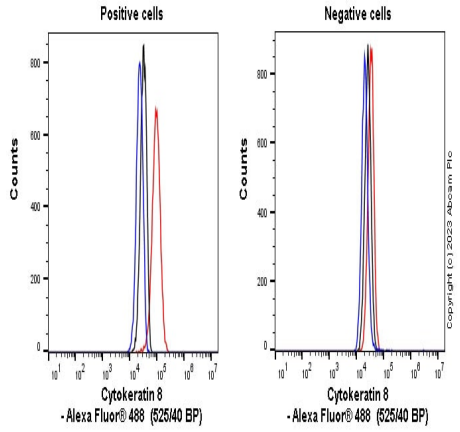
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 52 kDa (predicted molecular weight: 54 kDa).
IP		Use at an assay dependent concentration.
IHC-P	★★★★★ (1)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376**- Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-Fr		Use at an assay dependent concentration.

Target

Function	Together with KRT19, helps to link the contractile apparatus to dystrophin at the costameres of striated muscle.
Tissue specificity	Observed in muscle fibers accumulating in the costameres of myoplasm at the sarcolemma membrane in structures that contain dystrophin and spectrin. Expressed in gingival mucosa and hard palate of the oral cavity.
Involvement in disease	Cirrhosis
Sequence similarities	Belongs to the intermediate filament family.
Post-translational modifications	Phosphorylation on serine residues is enhanced during EGF stimulation and mitosis. Ser-74 phosphorylation plays an important role in keratin filament reorganization. O-glycosylated. O-GlcNAcylation at multiple sites increases solubility, and decreases stability by inducing proteasomal degradation. O-glycosylated (O-GlcNAcylated), in a cell cycle-dependent manner.
Cellular localization	Cytoplasm. Nucleus, nucleoplasm. Nucleus matrix.

Images



Flow Cytometry (Intracellular) - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

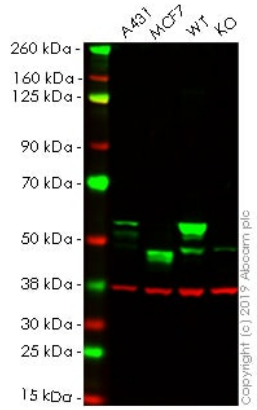
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53280**).

Flow cytometry overlay histogram showing left NIH3T3 positive cells and right negative Raw264.7 stained with **ab53280** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab53280**) (1x 10^6 in 100µl at 0.2µg/ml (1/11500)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Western blot - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

All lanes : Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (**ab53280**) at 1/10000 dilution

Lane 1 : A431 cell lysate
Lane 2 : MCF7 cell lysate
Lane 3 : Wild-type HeLa cell lysate
Lane 4 : KRT8 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

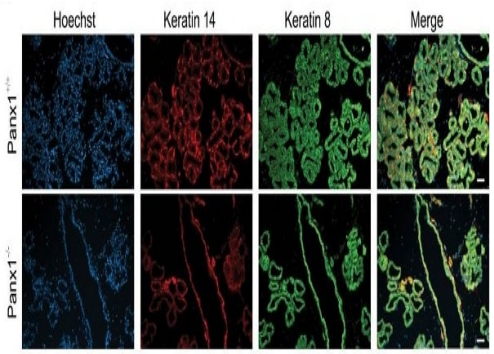
Performed under reducing conditions.

Predicted band size: 54 kDa
Observed band size: 52 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab53280**).

Lanes 1 - 4: Merged signal (red and green). Green - **ab53280** observed at 55 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab53280 was shown to react with Cytokeratin 8 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab255400** (knockout cell lysate **ab263785**) was used. Wild-type and Cytokeratin 8 knockout samples were subjected to SDS-PAGE. **ab53280** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 10000 (For unpurified use at 1/25,000 - 1/50,000) dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

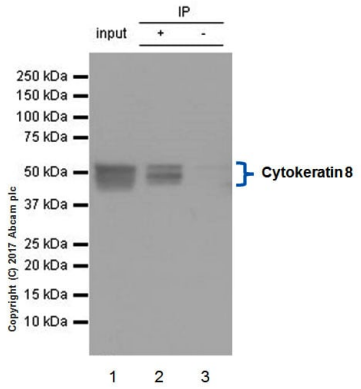
Image from Stewart M.K. et al PLoS One. 2016 Apr 21;11(4):e0154162. doi: 10.1371/journal.pone.0154162. eCollection 2016.

Panx1$^{-/-}$ mice have normal mammary gland epithelial differentiation at lactation

Immunofluorescent analysis of luminal epithelial marker keratin 8 (green) and myoepithelial marker keratin14 (red) revealed a similar staining pattern in Panx1$^{-/-}$ mice compared to control mice during lactation. Paraffin-embedded tissue samples.

Hoescht (blue) denotes nuclei. N = 6. Scale bars = 50 um.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53280**).



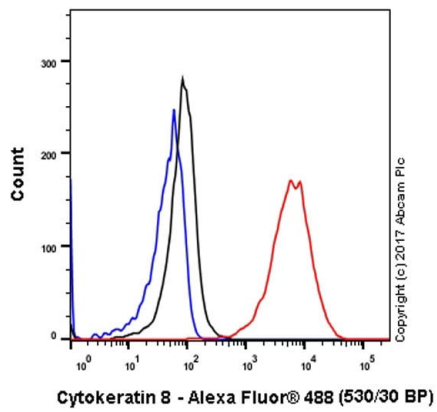
Immunoprecipitation - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

ab53280 (purified) at 1:20 dilution (0.2µg) immunoprecipitating Cytokeratin 8 in HeLa whole cell lysate.

Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate, 10µg
Lane 2 (+): ab53280 & HeLa whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of **ab53280** in HeLa whole cell lysate

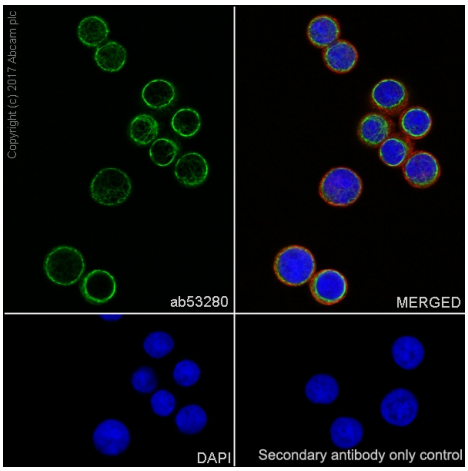
For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1:1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53280**).



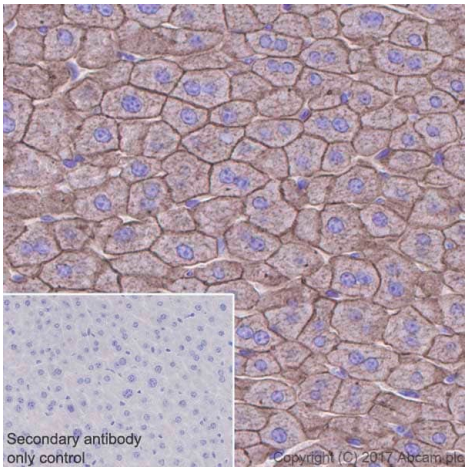
Flow Cytometry (Intracellular) - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Cytokeratin 8 with purified **ab53280** at 1/20 dilution (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53280**).



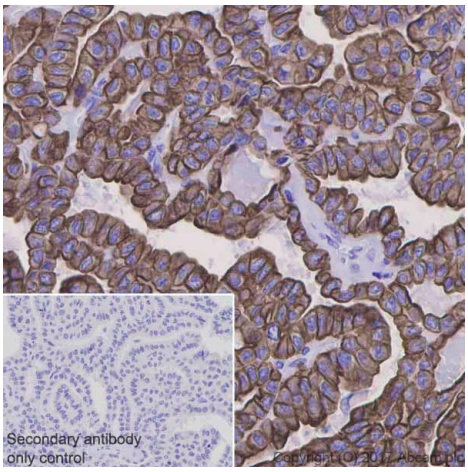
Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

Immunocytochemistry/ Immunofluorescence analysis of HT-29 (Human colorectal adenocarcinoma epithelial cell) cells labeling Cytokeratin 9 with Purified **ab53280** at 1:500 dilution. Cells were fixed in 100% Methanol and permeabilized with None. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor ® 594) 1:200 (2.5 µg/ml). **ab150077** Goat anti rabbit IgG(Alexa Fluor ® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53280**).



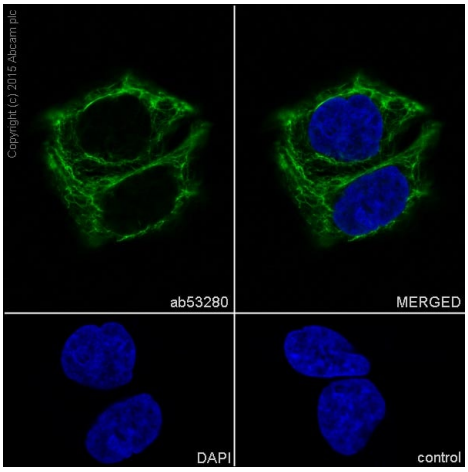
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse liver tissue sections labeling Cytokeratin 8 with Purified **ab53280** at 1:250 dilution. Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53280**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

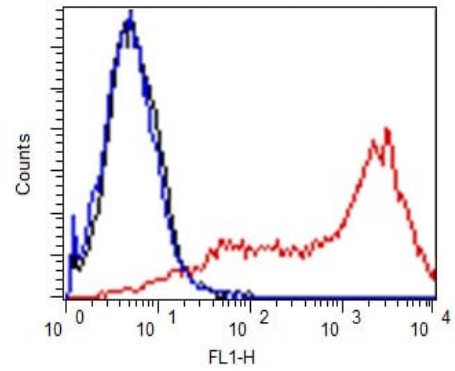
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thyroid carcinoma tissue sections labeling Cytokeratin 8 with Purified **ab53280** at 1:250 dilution. Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53280**).



Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

Immunocytochemistry/Immunofluorescence analysis of HeLa (human cervix adenocarcinoma) labelling Cytokeratin 8 with purified **ab53280** at 1/500. Cells were fixed with 4% Paraformaldehyde and permeabilised by 0.1% tritonX-100. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody (Ab150077). Nuclei counterstained with DAPI (blue).

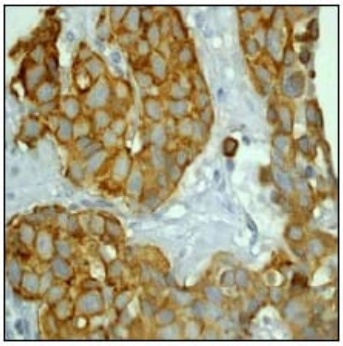
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53280**).



Flow Cytometry (Intracellular) - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

Overlay histogram showing HeLa cells stained with unpurified **ab53280** (red line). The cells were fixed with 2% PFA (room temperature, 30 min) and then permeabilized with 1% FACS permeabilizing solution for 30 min. The cells were then incubated in 3% FBS in 1X PBS followed by the antibody (**ab53280**, 1/20 dilution) for 1 hour at room temperature. The cells were then incubated for 30 min at room temperature with the secondary antibody. An isotype control antibody (black line) was used and an unlabelled sample (blue line) was also used as a control.

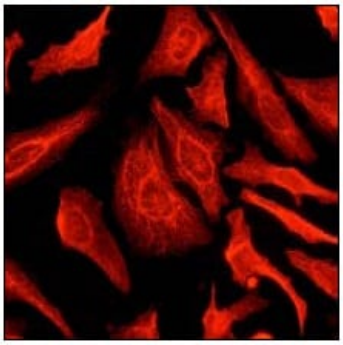
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53280**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

Unpurified **ab53280** (1:250) staining human Cyotkeratin 8 in human breast adenocarcinoma tissue by immunohistochemistry using paraffin embedded tissue.

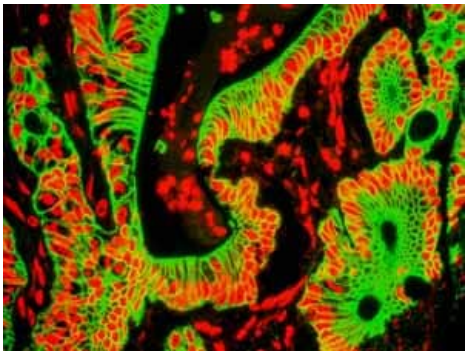
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53280**).



Immunocytochemistry/ Immunofluorescence - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

Immunofluorescent staining of HeLa cells using unpurified **ab53280** (1:100).

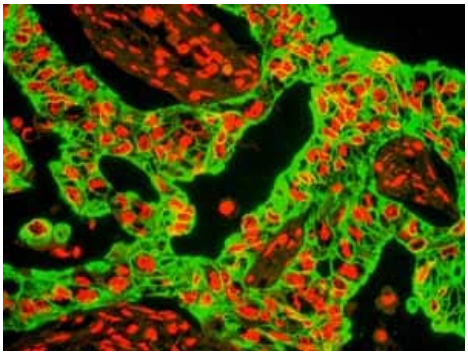
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53280**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

Fluorescent immunohistochemical analysis of paraffin-embedded human colonic adenocarcinoma tissue using unpurified **ab53280**. Green-CK8 red-PI.

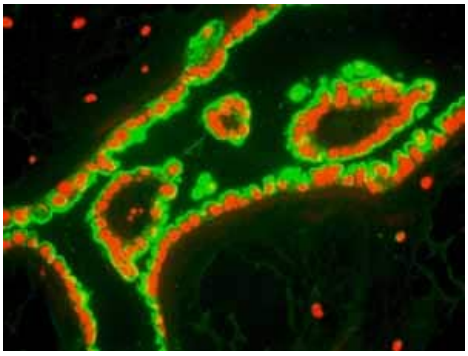
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53280**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

Fluorescent immunohistochemical analysis of paraffin-embedded human endometrial carcinoma tissue using unpurified **ab53280**. Green-CK8 red-PI.

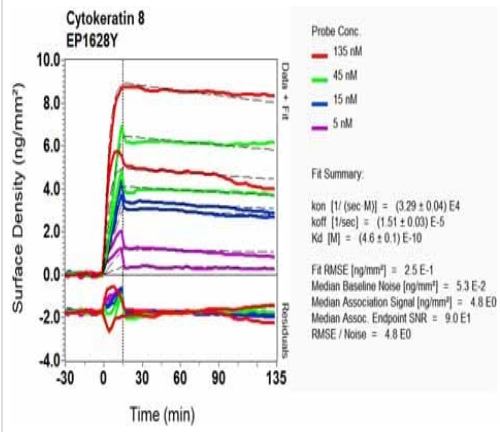
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53280**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

Fluorescent immunohistochemical analysis of paraffin-embedded human ovarian carcinoma tissue using unpurified **ab53280**. Green-CK8 red-PI.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53280**).



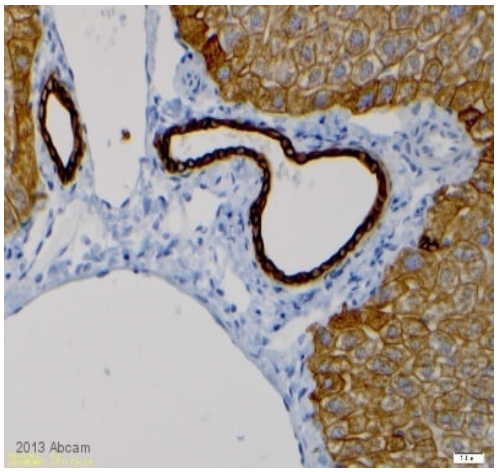
OI-RD Scanning - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53280**).

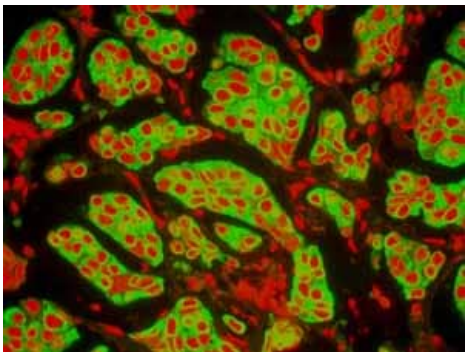


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

This image is courtesy of an anonymous Abreview.

Unpurified **ab53280** staining Cytokeratin 8 in Mouse liver tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formalin and blocked with 10% serum for 20 minutes at 23°C; antigen retrieval was by heat mediation in a citrate buffer. Samples were incubated with primary antibody (1/75 in TBS + 1% BSA) for 1 hour at 23°C. A Biotin-conjugated Goat anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody.

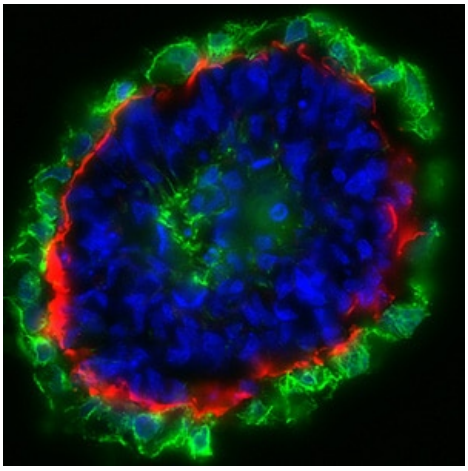
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab53280**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

This IHC data was generated using the same anti-Cytokeratin 8 antibody clone, EP1628Y, in a different buffer formulation (cat# **ab53280**).

Fluorescent immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using **ab53280**. Green-CK8 red-PI



Immunohistochemistry (Frozen sections) - Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

This image is courtesy of Dr. Shaohua Li

This IHC data was generated using the same anti-Cytokeratin 8 antibody clone, EP1628Y, in a different buffer formulation (cat# **ab53280**).

Image: Courtesy of Dr. Shaohua Li, UMDNJ-Robert Wood Johnson Medical School

Sample: mouse embryonic stem cell-differentiated embryoid bodies (EBs)

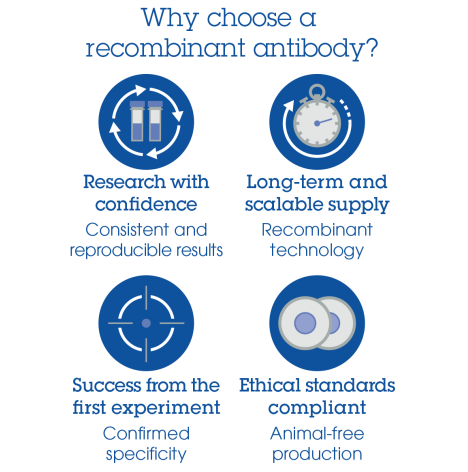
Preparation:

Fix in 3% PFA in PBS for 30 min at RT Incubate in 7.5% sucrose-PBS for 3h at RT Incubate in 15% sucrose-PBS at 4 degree Celsius overnight Embed the EBs in tissue-Tek OCT compound Cut frozen sections to 4-20 µm thickness

Primary antibody 1: Rabbit anti cytokeratin 8 (**ab53280**), 1:100

Primary antibody 2: Rat anti-perlecan, 1:100
Secondary antibody 1: Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488) pre-adsorbed (**ab150081**), 1:200

Secondary antibody 2: Goat polyclonal Secondary Antibody to Rat IgG - H&L (Cy5®) pre-adsorbed (**ab150081**), 1:200
Nuclei were counterstained with DAPI.



Anti-Cytokeratin 8 antibody [EP1628Y] - BSA and Azide free (ab217173)

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