

EpCAM Recombinant Polyclonal Antibody (22 HCLC)

Catalog Number710524

Product data sheet

Details		Species Reactivity	
Size	100 µg	Species reactivity	Human
Host/Isotope	Rabbit / IgG	Published species	Human
Class	Recombinant Polyclonal	Tested Applications	
Type	Antibody	ELISA (ELISA)	Dilution * Assay-Dependent
Clone	22 HCLC	Western Blot (WB)	1:500-1:5,000
Immunogen	proprietary	Immunocytochemistry (ICC/IF)	1:100-1:1,000
Conjugate	Unconjugated	Published Applications	
Form	Liquid	Western Blot (WB)	See 1 publications below
Concentration	0.5 mg/mL	* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.	
Purification	Protein A		
Storage buffer	PBS		
Contains	0.09% sodium azide		
Storage Conditions	Store at 4°C short term. For long term storage, store at -20°C, avoiding freeze/thaw cycles.		

Product specific information

This antibody is predicted to react with bovine, non-human primate, rabbit and ovine based on sequence homology. Recombinant rabbit polyclonal antibodies are unique offerings from Thermo Fisher Scientific. They are comprised of a selection of multiple different recombinant monoclonal antibodies, providing the best of both worlds - the sensitivity of polyclonal antibodies with the specificity of monoclonal antibodies - all delivered with the consistency only found in a recombinant antibody. While functionally the same as a polyclonal antibody - recognizing multiple epitope sites on the target and producing higher detection sensitivity for low abundance targets - a recombinant rabbit polyclonal antibody has a known mixture of light and heavy chains. The exact population can be produced in every lot, circumventing the biological variability typically associated with polyclonal antibody production.

Background/Target Information

Ep-CAM (epithelial adhesion molecule, epithelial specific antigen, ESA) is a transmembrane glycoprotein expressed in the epithelium with a molecular weight of approximately 40 kDa, which functions as an epithelial cell adhesion molecule. Ep-CAM functions as a homotypic calcium-independent cell adhesion molecule, and has a direct impact on cell cycle, proliferation and metabolism of epithelial cells and fibroblasts due to its ability to rapidly induce the proto-oncogene c-myc and the cell cycle regulating genes cyclin A and E. Ep-CAM mediates Ca2+-independent homotypic interactions. Formation of Ep-CAM-mediated adhesions have a negative regulatory effect on adhesions mediated by classic cadherins, which may have strong effects on the differentiation and growth of epithelial cells. Ep-CAM overexpression was suggested to be associated with enhanced epithelial proliferation. Ep-CAM is highly expressed in human carcinomas, and is a marker for tumors of epithelial lineage. Ep-CAM is expressed on baso-lateral cell surface in most simple epithelia and many carcinoma types. Also, Ep-CAM reportedly distinguishes adenocarcinomas from pleural mesotheliomas.

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EpCAM Antibody (710524) in WB

Western Blot was performed using Anti-EpCAM Recombinant Polyclonal Antibody (22 HCLC) (Product # 710524) and a 35 kDa band corresponding to Epithelial cell adhesion molecule was observed across cell lines tested. Membrane enriched extracts (30 µg lysate) of HCT 116 (Lane 1), Caco-2 (Lane 2), HT-29 (Lane 3), HeLa (Lane 4) and BJ (Lane 5) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0321BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (1:1000 dilution) and detected by chemiluminescence with Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:20000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Product # 34580).

EpCAM Antibody (710524)

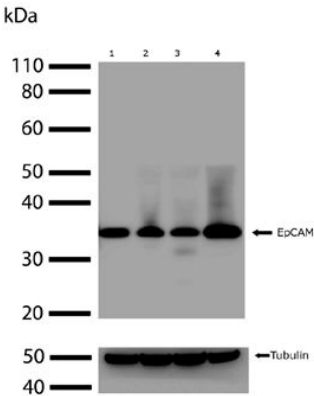
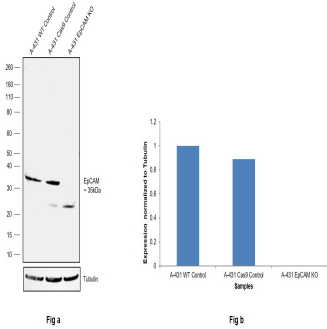
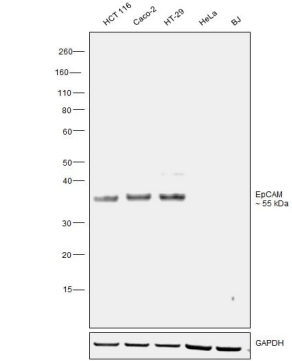
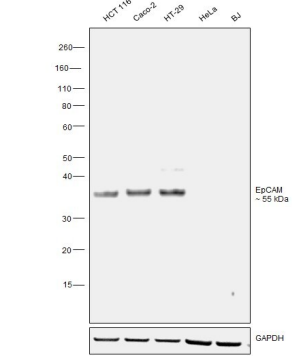
Antibody specificity was demonstrated by detection of differential basal expression of the target across cell lines tested owing to their inherent genetic constitution. Relative expression of Epithelial cell adhesion molecule was observed to be high in HT-29,HCT 116 and Caco-2 in comparison to no expression in HeLa and BJ using Anti-EpCAM Recombinant Polyclonal Antibody (22 HCLC) (Product # 710524) in Western Blot. {RE}

EpCAM Antibody (710524)

Antibody specificity was demonstrated by CRISPR-Cas9 mediated knockout of target protein. A loss of signal was observed for target protein inEpCAM KO cell line compared to control cell line using an EpCAM Recombinant Polyclonal Antibody (22 HCLC) (Product # 710524). {KO}

EpCAM Antibody (710524) in WB

Western blot analysis of endogenous EpCAM in 30 µg of HeLa (lane 1), MDAMB231 (lane 2), HEK-293 (lane 3), and COS-7 (lane 4) whole cell extracts using an EpCAM Recombinant Rabbit Polyclonal Antibody (Product # 710524) at a concentration of 2 µg/mL. A rabbit Anti-tubulin antibody was blotted as a loading control and the blots were developed using chemiluminescence (ECL) method with a Goat anti-Rabbit HRP secondary antibody (Product # G-21234). Results show a band at ~35kDa.



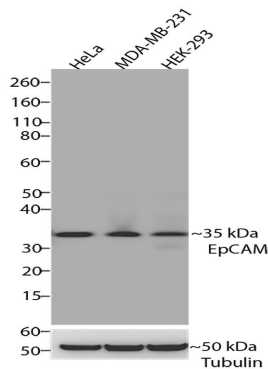
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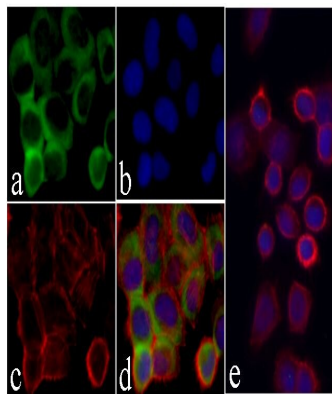
EpCAM Antibody (710524) in WB

Western blot analysis of EpCAM/CD326 was performed by loading 30 µg of HeLa, MDA-MB-231 and HEK-293 cell lysates using Novex®NuPAGE® 4-12% Bis-Tris gel (Product # NP0321BOX), XCell SureLock Electrophoresis System (Product # EI0002), Novex® Sharp Pre-Stained Protein Standard (Product # LC5800), and iBlot® Dry Blotting System (Product # IB21001). Proteins were transferred to a nitrocellulose membrane and blocked with 5% skim milk for 1 hour at room temperature. EpCAM/CD326 was detected at ~35 kDa using EpCAM/CD326 Recombinant Rabbit Polyclonal Antibody (Product # 710524) at a 1:1000 dilution in 2.5% skim milk at 4°C overnight on a rocking platform. Detection was performed using an HRP-conjugated Goat anti-Rabbit secondary antibody (Product # G-21234) at a 1:5000 dilution and chemiluminescent detection was performed using Pierce™ ECL Western blotting Substrate (Product # 32106).



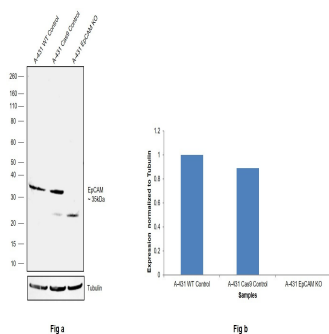
EpCAM Antibody (710524) in ICC/IF

Immunofluorescent analysis of EpCAM/CD326 was performed on 70% confluent log phase HeLa cells. The cells were fixed with 4% paraformaldehyde for 15 minutes, permeabilized with 0.25% Triton X-100 for 10 minutes, and blocked with 5% BSA for 1 hour at room temperature. The cells were labeled with EpCAM/CD326 Recombinant Rabbit Polyclonal Antibody (Product # 710524) at a dilution of 1:500 in 1% BSA and incubated for 3 hours at room temperature and then labeled with Alexa Fluor® 488 Goat anti-Rabbit IgG secondary antibody (Product # A-11008) at a dilution of 1:400 for 30 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Alexa Fluor 594 phalloidin (Product # A12381). Panel d is a merged image showing cytoplasmic localization and panel e is a control without primary antibody. The images were captured using a Nikon microscope at 20X magnification.



EpCAM Antibody (710524) in WB

Knockout of EpCAM was achieved by CRISPR-Cas9 genome editing using LentiArray™ Lentiviral sgRNA (Product # A32042, Assay ID CRISPR701274_LV) and LentiArray Cas9 Lentivirus (Product # A32064). Western blot analysis of EpCAM was performed by loading 30 µg of A-431 Wild Type (Lane 1), A-431 Cas9 (Lane 2) and A-431 EpCAM KO (Lane 3) membrane enriched extracts. The samples were electrophoresed using NuPAGE™ Novex™ 4-12% Bis-Tris Protein Gel (Product # NP0322BOX). Resolved proteins were then transferred onto a PVDF membrane (Product # LC2002) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with an EpCAM Recombinant Polyclonal Antibody (22 HCLC) (Product # 710524, 1:1,000 dilution) and Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:6,000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using SuperSignal™ West Dura Extended Duration Substrate (Product # 34076). Loss of signal upon CRISPR mediated knockout (KO) using the LentiArray™ CRISPR product line confirms that antibody is specific to EpCAM. An uncharacterized band was observed in CAS9 and EpCAM KO samples at ~25 kDa.



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PubMed References For EpCAM Recombinant Polyclonal Antibody (22 HCLC)

1 Western Blot References

Species / Dilution	Summary
	710524 was used in Western Blotting to develop a reproducible scaffold-free 3D organoid model to study neoplastic progression in breast cancer.
Human / Not Cited	Journal of cell communication and signaling (2019; 13: 129) "A reproducible scaffold-free 3D organoid model to study neoplastic progression in breast cancer." Author(s):Djomehri SI,Burman B,Gonzalez ME,Takayama S,Kleer CG PubMed Article URL: http://dx.doi.org/10.1007/s12079-018-0498-7

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