Human S100A4/p9Ka Rabbit Polyclonal Antibody

Cat# CY-P1026

50 µg (1.0 mg/mL x 50 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
	WB, E	H, M, R	9 kDa	Rabbit IgG

Background

Members of the S100 protein family are low molecular mass acidic proteins characterized by cell-type-specific expression and the presence of 2 EF-hand calcium-binding domains. The calgranulins are S100 proteins that are expressed in neutrophils, and are abundant in infiltrating monocytes and granulocytes under conditions of chronic inflammation.

Elevated levels of one S100 protein, S100A4, are closely associated with the process of metastasis in breast and other cancer cells in rodent animal models and in human cancer specimens. S100A4 or its mRNA is found at an elevated level in metastatic relative to non-metastatic rat (2) and mouse (3) tumor cell lines and benign relative to malignant human breast tumors (4). Elevation of the level of rat (5) or human (6) S100A4 in benign rat mammary tumor cells results in the acquisition of metastatic capability by some of the cells. In transgenic mouse models of breast cancer, elevated levels of S100A4 in neu oncogene-induced (7), or in murine mammary tumor virus-induced (8), benign mammary tumors yield lung metastases. In colorectal adenocarcinoma specimens, elevated levels of immunocytochemically detected S100A4 are associated with the more malignant carcinomatous regions of the primary tumors and with liver metastases (9). The precise interactions whereby S100A4 induces metastasis are not fully understood. Recently, methionine aminopeptidase 2 (10), S100A1 (11) and tumor suppressor p53 protein (12), have been demonstrated to interact physically and functionally with S100A4.

Specificity/Sensitivity: Human S100A4/p9Ka Antibody detects endogenous levels of S100A4 protein.

Source/Purification: Polyclonal antibody is produced by immunizing rabbit with a recombinant human S100A4 produced by *E. coli*. IgG is purified by immunoaffinity chromatography.

Recommended Antibody Dilutions: Western blotting: 0.5-1 µg/mL

Storage: S Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at -20°C.

Applications Key: WB: Western **IP:** Immunoprecipitation **IHC:** Immunohistochemistry **IC:** Immunocytochemistry **F:** Flow cytometry **E:** ELISA **FP:** Fluorescence Polarization assay
Species Cross-Reactivity Key: H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Mi:** mink **C:** chicken **X:** *Xenopus* **Z:** zebra fish **All:** all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

General References:
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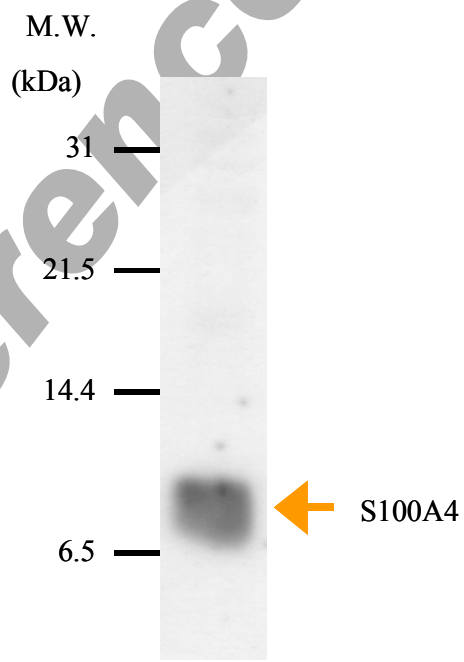
Fig. 1. Western blot analysis of Human S100A4 using HeLa cell lysate



Western Immunoblotting Protocol

Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
SDS Sample Buffer (1X): 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
Blocking Buffer: 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 mL, add 15 mL 10X TBS to 135 mL water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 mL Tween-20 (100%).
10X TBS (Tris-buffered saline): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween-20 with 5% blocking agent for 20 mL, add 2 mL 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µL Tween-20 (100%).
Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)
Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20
Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS, aspirate.
3. Lyse cells by adding 1X SDS Sample Buffer (100 µL per well of 6-well plate or 500 µL per plate of 10 cm2 plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µL sample to 95–100°C for 5 minutes, cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µL onto 17.5 % SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose membrane.

Membrane Blocking and Antibody Incubations

Note: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 mL TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 mL of Blocking Buffer for 1 hour at room temperature.
3. Wash 3 times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 mL Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
5. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:3000 in 10 mL of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 mL of TBS/T.

Detection of Proteins

1. Incubate membrane with 4 mL ECL™ with gentle agitation for 1 minute at room temperature.
2. Drain membrane of excess developing solution, do not let dry, wrap in plastic wrap and expose to x-ray film. An initial ten-second exposure should indicate the proper exposure time.

Related Products

*** CircuLex S100A13 ELISA Kit: Cat# CY-8057**
*** CircuLex S100A12 ELISA Kit: Cat# CY-8058**
*** CircuLex S100P ELISA Kit: Cat# CY-8060**
*** CircuLex S100A8-MRP8 ELISA Kit: Cat# CY-8061**
*** CircuLex S100A9-MRP14 ELISA Kit: Cat# CY-8062**
*** CircuLex S100A11 ELISA Kit: Cat# CY-8063**
*** CircuLex S100A14 ELISA Kit: Cat# CY-8064**
*** CircuLex S100A7/Psoriasin ELISA Kit: Cat# CY-8073**
*** CircuLex S100A4 ELISA Kit Ver.2: Cat# CY-8086**

*** Anti-Human S100A3 (Clone YK-3E3): Cat# CY-M1039**
*** Anti-Human S100A4 (p9Ka): Cat# CY-P1026**
*** Anti-Human S100P: Cat# CY-P1028**
*** Anti-Human S100A10: Cat# CY-P1033**
*** Anti-Human S100A16: Cat# CY-P1034**
*** Anti-Human S100A3: Cat# CY-P1039**
*** Anti-Human S100A2: Cat# CY-P1040**

*** Human S100B: Cat# CY-R2250**
*** Human S100A1: Cat# CY-R2251**
*** Human S100A2: Cat# CY-R2252**
*** Human S100A3: Cat# CY-R2253**
*** Human S100A4: Cat# CY-R2254**
*** Human S100A5: Cat# CY-R2255**
*** Human S100A6: Cat# CY-R2256**
*** Human S100A7: Cat# CY-R2257**
*** Human S100A8: Cat# CY-R2258**
*** Human S100A9: Cat# CY-R2259-G**
*** Human S100A9: Cat# CY-R2259-H**
*** Human S100A10: Cat# CY-R2260**
*** Human S100A12: Cat# CY-R2262-G**
*** Human S100A12: Cat# CY-R2262-H**
*** Human S100A13: Cat# CY-R2263**
*** Human S100A14: Cat# CY-R2264**
*** Human S100A16: Cat# CY-R2266**
*** Human S100P: Cat# CY-R2267**
*** Human S100A11: Cat# CY-R2269**

*** Human S100A1 Low Endotoxin: Cat# CY-R2451**
*** Human S100A3 Low Endotoxin: Cat# CY-R2453**
*** Human S100A4 Low Endotoxin: Cat# CY-R2454**
*** Human S100A7 Low Endotoxin: Cat# CY-R2457**
*** Human S100A8 Low Endotoxin: Cat# CY-R2458**

*** Human S100A9 Low Endotoxin: Cat# CY-R2459-G**
*** Human S100A11 Low Endotoxin: Cat# CY-R2461**
*** Human S100A12 Low Endotoxin: Cat# CY-R2462-G**
*** Human S100A14 Low Endotoxin: Cat# CY-R2464**
*** Human S100P Low Endotoxin: Cat# CY-R2467**

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