CircuLex™

Human S100A10 Rabbit Polyclonal Antibody

50 µg (0.5 mg/mL x 100 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
	WB	H	11 kDa	Rabbit IgG

Background

S100A10, a member of the S100 family of Ca2+-binding proteins, is a dimeric protein composed of two 11-kDa subunits. The protein is cytosolic when present as a dimer. Typically, S100A10 is found in most cells bound to annexin A2 as the heterotetrameric (S100A10)2-(annexin A2)2 complex, AIIt, in a calcium-independent manner (1). The formation of AIIt results in the translocation of S100A10 to the plasma membrane (2-4). S100A10 has been shown to regulate plasma membrane ion channels (5, 6) as well as cytosolic phospholipase A2 (7). In addition to an intracellular distribution, it has also been established that the heterotetrameric form of S100A10 is present on the extracellular surface of many cells (8–14). Extracellularly, the S100A10 subunit functions as a plasminogen receptor (15, 16). The penultimate and ultimate carboxyl-terminal lysines of this subunit bind tPA and plasminogen (17) and regulate the stimulation of tPA-dependent plasminogen activation (18).

Specificity/Sensitivity: Human S100A10 Antibody detects endogenous levels of S100A10 protein.

Source/Purification: Polyclonal antibody is produced by immunizing rabbit with a recombinant human S100A10 produced by *E. coli*. IgG is purified by immunoaffinity chromatography.

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

Storage: Supplied in 10 mM phosphate buffer (pH 7.5), 150 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.

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General References:

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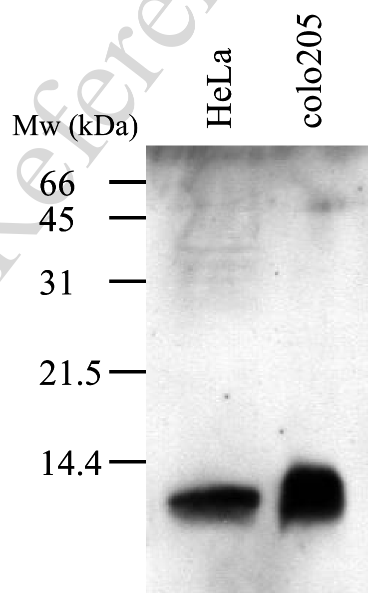
Fig. 1. Western blot analysis of Human S100A10



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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 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.

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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 Human S100A4 (p9Ka) Rabbit Polyclonal antibody Cat# CY-P1026
*CircuLex Human S100A12 (EN-RAGE) Rabbit Polyclonal antibody Cat# CY-P1027
*CircuLex Human S100P Rabbit Polyclonal antibody Cat# CY-P1028

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