

# Human S100A6 Rabbit Polyclonal Antibody

Cat# CY-P1042

50 µg (1.0 mg/mL x 50 µL)

Clone Name	Applications	Species	Cross-Reactivity	Molecular Wt.	Source Isotype
-	WB		H	10.5-11 kDa	Rabbit IgG

## Background

S100A6 also known as calcyclin, a small acidic protein whose molecular weight is about 10.5 kDa, belongs to the S100 calcium-binding protein family (1). These family members share a common S100 calcium-binding motif and are involved in several regulatory functions that include protein phosphorylation, some enzyme activities, the dynamics of cytoskeletal components, transcription factors, and Ca<sup>2+</sup> homeostasis, and also cell proliferation and differentiation (2, 3).

S100A6 is most abundantly expressed in fibroblasts and epithelial cells (4) and has also been found in some neurons, astrocytes, smooth muscle cells, cardiac myocytes, platelets and lymphocytes. S100A6 is also expressed by osteoblasts and up-regulated markedly during osteoblast differentiation; however, its roles in bone physiology are unknown (5). In addition, S100A6 is overexpressed in several tumor cells with high metastatic activity, e.g. melanoma (6). Therefore S100A6 may also be involved in tumorigenesis.

The general function of S100A6 remains unclear, but evidence suggests that it is involved in cell cycle regulation (7, 8) and exocytosis (9, 10), and in the regulation of heat shock proteins and cytoskeletal dynamics. Interestingly overexpression of S100A6 has also been observed in patients suffering from Alzheimer disease or amyotrophic lateral sclerosis (11, 12).

**Specificity/Sensitivity:** The Human S100A6 Antibody detects endogenous levels of S100A6 protein.

**Source/Purification:** The antibody is produced by immunizing rabbit with a recombinant human S100A6, corresponding full length of human S100A6, expressed in *E. coli*. IgG is purified by immunoaffinity chromatography.

**Recommended Antibody Dilutions:** Western blotting: 1-2 µg/mL.

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

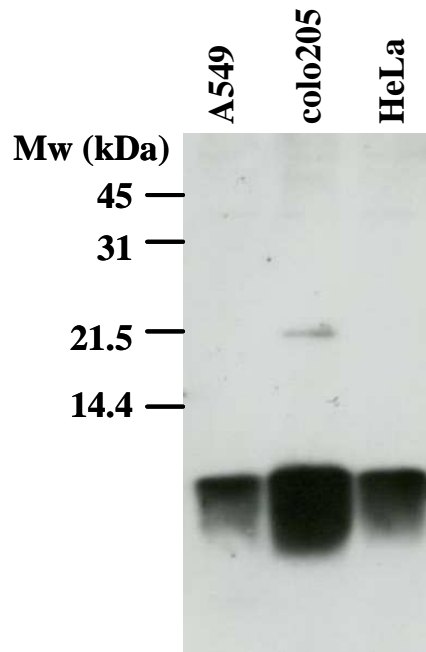
**Applications Key:** WB: Western IP: Immunoprecipitation IHC: Immunohistochemistry IC: Immunocytochemistry F: Flow cytometry E: ELISA FP: Fluorescence Polarization assay

**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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8. Calabretta B, Venturelli D, Kaczmarek L, Narni F, Talpaz M, Anderson B, Beran M, Baserga R.; Altered expression of G1-specific genes in human malignant myeloid cells. *Proc Natl Acad Sci U S A.* 83, 1495-1498, 1986
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11. Boom A, Pochet R, Authelet M, Pradier L, Borghgraef P, Van Leuven F, Heizmann CW, Brion J-P. ; Astrocytic calcium/zinc binding protein S100A6 over expression in alzheimer's disease and in PS1/APP transgenic mice models. *Biochim. Biophys. Acta* 1742, 161-168, 2004
12. Hoyaux D., Boom A., Van den Bosch L., Belot N., Martin J.J., Heizmann C.W., Kiss R., Pochet R.; S100A6 overexpression within astrocytes associated with impaired axons from both ALS mouse model and human patients. *J. Neuropathol. Exp. Neurol.* 61, 736-744, 2002

Fig.1 WB analysis of endogenous human S100A6 protein



### Western blotting 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  $\mu$ L 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  $\mu$ L per well of 6-well plate or 500  $\mu$ L per plate of 10 cm<sup>2</sup> 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  $\mu$ L sample to 95–100°C for 5 minutes, cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20  $\mu$ L 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<sup>2</sup>) 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 (1:3,000-5,000 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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