

#2213 Store at -20°C

Glut4 (1F8) Mouse mAb

✓ 100 µl (10 Western mini-blot)



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This product is for *in vitro* research use only and is not intended for use in humans or animals. This product is not intended for use as a therapeutic or in diagnostic procedures.

Applications W	Species Cross-Reactivity* M, R	Molecular Wt. 45 kDa	Isotype Mouse IgG**
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Background: A group of related glucose transporters (Glut1-5 and 7) mediate the facilitated diffusion of glucose in nonepithelial mammalian tissues. Within insulin-responsive tissues such as muscle and fat, Glut1 contributes to basal glucose uptake while Glut4 is responsible for insulin-stimulated glucose transport (1-3). Glut4 is a 12-transmembrane domain protein that facilitates glucose transport in the direction of the glucose gradient. This transporter localizes to intracellular organelles (endosomes) in unstimulated cells and translocates to the cell surface following insulin stimulation (1,2,4). Translocation of Glut4 is dependent on Akt, which may act by phosphorylating AS160, a RabGAP protein involved in membrane trafficking (5).

Specificity/Sensitivity: Glut4 (1F8) Mouse mAb detects endogenous levels of total Glut4 protein. It does not cross-react with other related proteins.

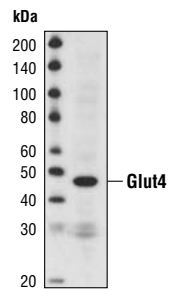
Source/Purification: Monoclonal antibodies are produced by immunizing mice with partially purified intracellular glucose transporter-containing vesicles obtained from rat adipocytes.

Selected Application References:

Gross, D.N. et al. (2004) Glut4 storage vesicles without Glut4: transcriptional regulation of insulin-dependent vesicular traffic. *Mol. Cell. Biol.* 24, 7151-7162.

Fischer, Y. et al. (1997) Insulin-induced recruitment of glucose transporter 4 (GLUT4) and GLUT1 in isolated rat cardiac myocytes. Evidence of the existence of different intracellular GLUT4 vesicle populations. *J. Biol. Chem.* 272, 7085-7092.

James, D.E. et al. (1988) Insulin-regulatable tissues express a unique insulin-sensitive glucose transport protein. *Nature* 333, 183-185.



Western blot analysis of extracts from H-4-II-E cells using Glut4 (1F8) Mouse mAb.

Background References:

- (1) Marette, A. et al. (1992) *Am. J. Physiol.* 263, C443-C452.
- (2) Slot, J.W. et al. (1991) *Proc. Natl. Acad. Sci. USA* 88, 7815-7819.
- (3) James, D.E. et al. (1989) *Nature* 338, 83-87.
- (4) Barrett, M.P. et al. (1999) *Curr. Opin. Cell Biol.* 11, 496-502.
- (5) Zeigerer, A. et al. (2004) *Mol. Biol. Cell* 15, 4406-4415.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by Western blot.
**Anti-mouse secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:
Western blotting 1:1000

Companion Products:

Glut4 Antibody #2299
IRS-1 Antibody #2382
Phospho-IRS-1 (Ser1101) Antibody #2385
Phospho-IRS-1 (Ser307) Antibody #2381
IRS-1 (59G8) Rabbit mAb #2390
Phospho-IRS-1 (Ser302) Antibody #2384
Phototope®-HRP Western Blot Detection System, Anti-mouse IgG, HRP-linked Antibody #7072
Anti-mouse IgG, HRP-linked Antibody #7076
Prestained Protein Marker, Broad Range (Premixed Format) #7720
Biotinylated Protein Ladder Detection Pack #7727
20X LumiGLO® Reagent and 20X Peroxide #7003

IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

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Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E—ELISA E-P—ELISA Peptide

Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebra fish B—bovine

Dg—dog Pg—pig Sc—S. cerevisiae All—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

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Western Immunoblotting Protocol (Primary Antibody Incubation In Milk)

For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

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

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 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
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** 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).
- Nonfat Dry Milk (weight to volume [w/v])
- 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%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope®-HRP Western Blot Detection System #7072:** Includes biotinylated protein ladder, secondary anti-mouse (#7076) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

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

NOTE: CST recommends loading prestained molecular weight marker (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm²) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.