

#3194 Store at -20°C

IRS-1 (L3D12) 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.

| Applications | Species Cross-Reactivity | Molecular Wt. | Source | Isotype |
|--------------|--------------------------|---------------|--------|---------|
| W | H, M, R | 180 kDa | Mouse | IgG |

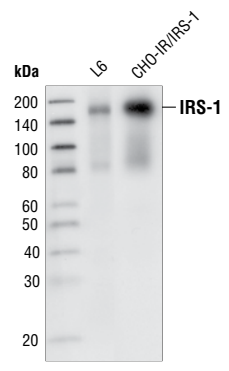
Background: Insulin receptor substrate 1 (IRS-1) is one of the major substrates of the insulin receptor kinase (1). IRS-1 contains multiple tyrosine phosphorylation motifs that serve as docking sites for SH2-domain-containing proteins, which mediate the metabolic and growth-promoting functions of insulin (2–4). IRS-1 also contains over 30 potential serine/threonine phosphorylation sites. Ser307 of IRS-1 is phosphorylated by JNK (5) and IKK (6) and Ser789 is phosphorylated by SIK-2, a member of AMPK family (7). The phosphorylation of Ser612 and Ser636/639 is mediated by the PKC and mTOR pathways, respectively (8,9) and phosphorylation at Ser1101 is mediated by PKC ζ (10), resulting in an inhibition of insulin signaling in the cell, suggesting a potential mechanism for insulin resistance in some models of obesity.

Specificity/Sensitivity: IRS-1 (L3D12) Mouse mAb detects endogenous levels of total IRS-1.

Source/Purification: Monoclonal antibodies are produced by immunizing mice with a synthetic peptide (KLH-coupled) derived from the sequence surrounding Ser612 of human IRS-1.

Background References:

- (1) Sun, X.J. et al. (1991) *Nature* 352, 73–77.
- (2) Sun, X.J. et al. (1992) *J. Biol. Chem.* 267, 22662–22672.
- (3) Myers Jr., M.G. et al. (1993) *Endocrinology* 132, 1421–1430.
- (4) Wang, L.M. et al. (1993) *Science* 261, 1591–1594.



Western blot analysis of cell lysates from L6 and CHO cells overexpressing IRS-1 and insulin receptors, using IRS-1 (L3D12) Mouse mAb.

- (5) Rui, L. et al. (1997) *J. Clin. Invest.* 107, 181–189.
- (6) Gao, Z. et al. (2002) *J. Biol. Chem.* 277, 48115–48121.
- (7) Horike, N. et al. (2003) *J. Biol. Chem.* 278, 18440–18447.
- (8) Ozes, O.N. et al. (2001) *Proc. Natl. Acad. Sci. USA* 98, 4640–4645.
- (9) De Fea, K. and Ruth, R.A. (1997) *Biochemistry* 36, 12939–12947.
- (10) Li, Y. et al. (2004) *J. Biol. Chem.* 279, 45304–45307.

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.

Recommended Antibody Dilutions:
Western Blotting 1:1000

Companion Products:

- Phospho-IRS-1 (Ser307) Antibody #2381
- IRS-1 Antibody #2382
- Phospho-IRS-1 (Ser302) Antibody #2384
- Phospho-IRS-1 (Ser1101) Antibody #2385
- Phospho-IRS-1 (Ser612) Antibody #2386
- Phospho-IRS-1 (Ser636/639) Antibody #2388
- Phospho-IRS-1 (Ser789) Antibody #2389
- 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.

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

Western Immunoblotting Protocol (Primary Ab 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)
- Bovine Serum Albumin (BSA)
- 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.