Technical Data Sheet

PE Mouse anti-IRS-1 (pY896)

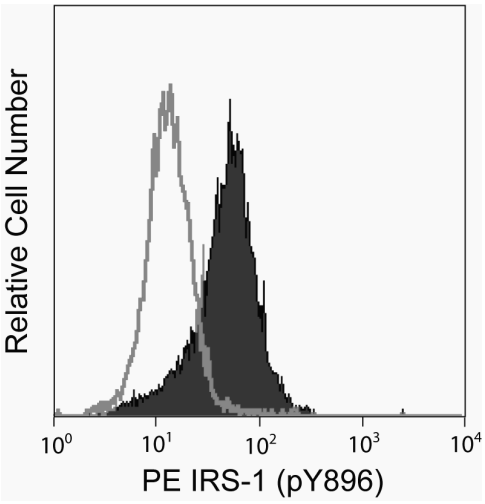
Product Information

Material Number:	**558440**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	K9-211
Immunogen:	Phosphorylated Human IRS-1 Peptide
Isotype:	Mouse (BALB/c) IgG2a, κ
Reactivity:	Confirmed: Human Predicted: Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The IRS (*I*nsulin *R*eceptor *S*ubstrate) proteins IRS-1, IRS-2, IRS-3, and IRS-4 are major substrates of the insulin receptor and the insulin-like growth factor-1 (IGF-1) receptor tyrosine kinases. IRS proteins contain an N-terminal pleckstrin homology (PH) domain, a phosphotyrosine-binding (PTB) domain, and multiple tyrosine phosphorylation sites in the C-terminus. The IRS-1 protein is widely expressed and, along with IRS-2, mediates somatic growth and carbohydrate metabolic responses to insulin. Following insulin receptor ligation, IRS-1 binds to the juxtamembrane region of the receptor via the PH and PTB domains and is tyrosine phosphorylated, which facilitates its interaction with SH2 domain-containing signaling proteins. Specifically, the phosphorylated tyrosine 896 (pY896) of human IRS-1 is a major binding site for the GRB2 (*G*rowth-factor *R*eceptor-*B*ound protein *2*) adaptor protein. After IRS-1 activation, negative and positive feedback regulates dephosphorylation of its tyrosine sites, which ultimately regulates the magnitude and/or duration of the downstream pleiotropic responses to insulin and IGF-1.

The K9-211 monoclonal antibody recognizes pY896 of human IRS-1. The orthologous phosphorylation sites of mouse and rat IRS-1 are Y891 and Y895, respectively.



Analysis of IRS-1 (pY896) in transformed human epithelial cells. *Serum-starved 293 fetal kidney cells were either stimulated for 2 minutes with 100 nM IGF-I (Cat. No. 354037, shaded histogram) or unstimulated (open histogram). The cells were fixed (BD™ Phosflow Fix Buffer I, Cat. No. 557870) for 10 minutes at 37°C, then permeabilized (BD™ Phosflow Perm Buffer III, Cat. No. 558050) on ice for at least 30 minutes, and then stained with PE Mouse anti-IRS-1 (pY896). Flow cytometry was performed on a BD™ FACSCalibur flow cytometry system.*

Preparation and Storage

The antibody was conjugated with R-PE under optimum conditions, and unconjugated antibody and free PE were removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Tested

Suggested Companion Products

Catalog Number	Name	Size	Clone
557870	Fix Buffer I	250 ml	(none)
558050	Perm Buffer III	125 ml	(none)

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Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
2. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

References

Burks DJ, White MF. IRS proteins and β-cell function. *Diabetes.* 2001; 50(S1):S140-S145. (Biology)

Gual P, Le Marchand-Brustel Y, Tanti JF. Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. *Biochimie.* 2005; 87:99-109. (Biology)

Paz K, Liu YF, Shorer H, et al. Phosphorylation of insulin receptor substrate-1 (IRS-1) by protein kinase B positively regulates IRS-1 function. *J Biol Chem.* 1999; 274(40):28816-28822. (Biology)

Ward CW, Gough KH, Rashke M, Wan SS, Tribbick G, Wang J. Systematic mapping of potential binding sites for Shc and Grb2 SH2 domains on insulin receptor substrate-1 and the receptors for insulin, epidermal growth factor, platelet-derived growth factor, and fibroblast growth factor. *J Biol Chem.* 1996; 271(10):5603-5609. (Biology)

White MF. IRS proteins and the common path to diabetes. *Am J Physiol Endocrinol Metab.* 2002; 283:E413-E422. (Biology)

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