Revision 2

Store at +4C

#7214

PathScan® Total IRS-1 Sandwich ELISA Kit

Cell Signaling TECHNOLOGY®

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UniProt ID: #P35568 **Entrez-Gene Id:** #3667

For Research Use Only. Not for Use in Diagnostic Procedures.

Product Includes	Product #	Quantity	Color	Storage Temp
Anti-rabbit IgG, HRP-linked Antibody (ELISA Formulated)	83876	11 ml	Red	+4C
TMB Substrate	7004	11 ml	Colorless	+4C
STOP Solution	7002	11 ml	Colorless	+4C
Sealing Tape	54503	2 ea		+4C
ELISA Wash Buffer (20X)	9801	25 ml	Colorless	+4C
Cell Lysis Buffer (10X)	9803	15 ml	Yellowish	-20C

Kit contents scale proportionally with size, except sealing tape.
Example: The V1 kit contains 5X the listed quantities above, but will exclude the sealing tape.

The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

Description

CST's PathScan® Total IRS-1 Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects transfected IRS-1 protein. An IRS-1 Mouse mAb #3194* has been coated onto the microwells. After incubation with cell lysates, both phospho- and nonphospho-IRS-1 proteins are captured by the coated antibody. Following extensive washing, IRS-1 Rabbit mAb #2397* is added to detect both the captured phospho- and nonphospho-IRS-1 protein. Anti-rabbit IgG, HRP-linked Antibody #7074* is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of optical density for this developed color is proportional to the quantity of total IRS-1 protein.
* Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity

CST's PathScan® Total IRS-1 Sandwich ELISA Kit #7214 detects IRS-1 protein. As shown in Figure 1, using PathScan® Phospho-IRS-1 (Ser612) ELISA Kit #7213, a significant induction of phospho-IRS-1 (Ser612) is detected in CHO-IR/IRS-1 cells treated with insulin. However, the levels of total IRS-1 (phospho and nonphospho) detected by PathScan® Total IRS-1 Sandwich ELISA Kit #7214 remain unchanged. In Figure 3, Western blot analysis of protein captured in the IRS-1 mouse mAb #3194 coated microwell shows a single band corresponding to the IRS-1 protein. In 3T3 cells, IRS-1 protein also can be detected by this kit (data not shown). This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

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 that 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) while Ser789 is phosphorylated by SIK-2, a member of the AMPK family (7). The PKC and mTOR pathways mediate phosphorylation of IRS-1 at Ser612 and Ser636/639, respectively (8,9). Phosphorylation of IRS-1 at Ser1101 is mediated by PKCθ and results in an inhibition of insulin signaling in the cell, suggesting a potential mechanism for insulin resistance in some models of obesity (10).

Background References

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