Revision 5

Store at -20C

#51367

4E-BP1 Control Cell Extracts

Controls for 10 western blots

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Product Includes	Product #	Quantity
4E-BP1 Control Cell Extracts (MCF7 untreated)	64125	100 µl
4E-BP1 Control Cell Extracts (MCF7 +insulin)	87570	100 µl

Description

Nonphosphorylated 4E-BP1 Control Cell Extracts: Total cell extracts from MCF7 cells, serum-starved overnight then amino acids starved for 1 hour to serve as a negative control. Supplied in SDS Sample Buffer.

Phosphorylated 4E-BP1 Control Cell Extracts: Total cell extracts from MCF7 cells, serum-starved overnight then amino acids starved for 1 hour, followed by adding back amino acids for 1 hour and treating with 100 nM insulin for 30 min to serve as a positive control. Supplied in SDS Sample Buffer.

Storage

Store at -20°C. Supplied in 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.

Background

Translation repressor protein 4E-BP1 (also known as PHAS-1) inhibits cap-dependent translation by binding to the translation initiation factor eIF4E. Hyperphosphorylation of 4E-BP1 disrupts this interaction and results in activation of cap-dependent translation (1). Both the PI3 kinase/Akt pathway and FRAP/mTOR kinase regulate 4E-BP1 activity (2,3). Multiple 4E-BP1 residues are phosphorylated *in vivo* (4). While phosphorylation by FRAP/mTOR at Thr37 and Thr46 does not prevent the binding of 4E-BP1 to eIF4E, it is thought to prime 4E-BP1 for subsequent phosphorylation at Ser65 and Thr70 (5).

Directions for Use

Boil for 3 minutes prior to use. Load 10 µl of phosphorylated and nonphosphorylated 4E-BP1 Control Cell Extracts per lane.

Background References

1. Pause, A. et al. (1994) *Nature* 371, 762-7.
2. Brunn, G.J. et al. (1997) *Science* 277, 99-101.
3. Gingras, A.C. et al. (1998) *Genes Dev* 12, 502-13.
4. Fadden, P. et al. (1997) *J Biol Chem* 272, 10240-7.
5. Gingras, A.C. et al. (1999) *Genes Dev* 13, 1422-37.

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