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Certificate of Analysis

Hsp70

(Recombinant protein expressed in Sf21 insect cells)
Item # 23-052, 23-052-K, 23-052M
Parent Lot # D11PP030N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: *N*-terminal c-Myc, 6His-tagged, recombinant human Hsp70 full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

Purity 78% by SDS-PAGE and Coomassie blue staining. MW = 75kDa.

Formulation: 1.499mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

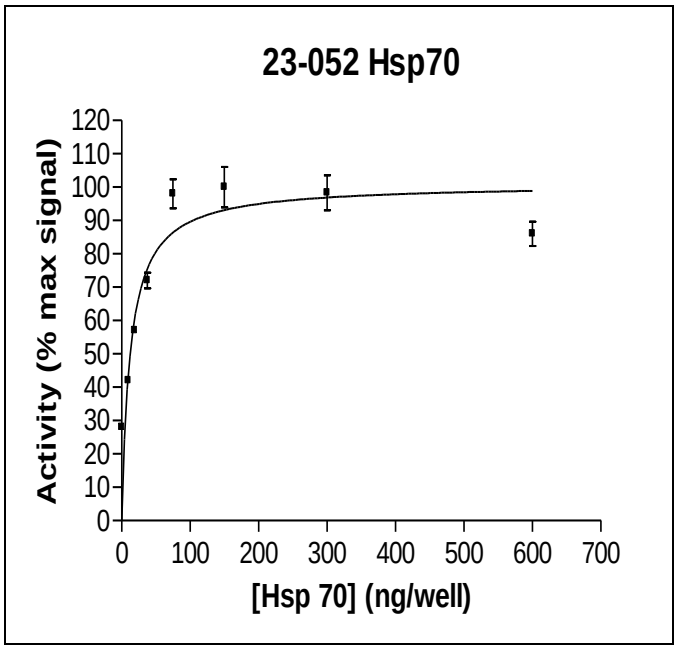
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

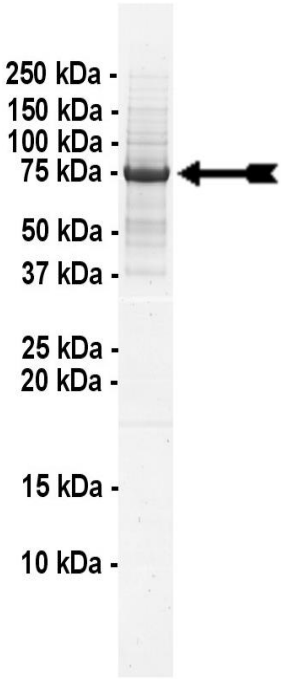
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Assay: This enzyme was titrated in a ubiquitination assay and the results normalised against the maximum signal.



Protein Identity: Confirmed identity as Hsp70 by mass spectrometry.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Hsp70.

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Assay Protocol

Reagents:

1. **UBE1, active (Item # 23-021)**
2. **UbcH13/Uev1A, active (Item # 23-051)**
3. **CHIP, active (Item # 23-050)**
4. **Hsp70 (Item # 23-052)**
5. **1x Reaction Buffer**
6. **Biotinylated-Ubiquitin**
7. **Stop Solution**

Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH7.5, 0.01% Tween 20, 5mM $MgCl_2$).

Hsp70 is incubated with 25mM MOPS pH7.5, 0.01% Tween 20, 5mM $MgCl_2$, 10µM ATP, 10nM UBE1, 1000nM UbcH13/Uev1A, 0.075µg CHIP and 2µM biotinylated-ubiquitin. The reaction is initiated with the addition of biotinylated-ubiquitin. After 30 minutes at room temperature the reaction is terminated by the addition of 25mM MOPS pH7.5 containing 125mM EDTA, 150mM NaCl, and 0.05% Tween 20. Reaction products are separated by capture onto a microplate coated with anti-c-Myc antibody and washing with PBS containing 0.05% Tween-20. Ubiquitination of Hsp70 is measured by detection of bound ubiquitin via electrochemiluminescence.

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Hsp70 Information

Protein human Hsp70

Accession number GenBank BC002453

Alternative Names Heat shock 70 kDa protein 1A/1B, HSPA1A, HSPA1B

Key Facts Hsp70 proteins are central components of the cellular network of molecular chaperones. They assist in a wide range of processes, including the folding and assembly of newly synthesized proteins, refolding of misfolded and aggregated proteins, membrane translocation of organellar and secretory proteins, and control of the activity of regulatory proteins. The cellular levels of Hsp70 are thought to be at least partially regulated by the E3 ubiquitin ligase CHIP, which also targets a broad range of Hsp70 chaperone substrates for proteosomal degradation.
Consistent with its diverse activities, genetic and biochemical studies have implicated Hsp70 in a range of diseases including cancer, neurodegeneration, allograft rejection and infection.

Related Products Item # 23-021 UBE1, active, Item # 23-051 UbcH13/Uev1A, active, Item # 23-050 CHIP, active

Selected References

Mayer M. P and Bukau B. Hsp70 Chaperones: Cellular Functions and Molecular Mechanism. Cell Mol Life Sci., *62*: 670-684, 2005

Qian S. B. *et al.,* CHIP-Mediated Stress Recovery by Sequential Ubiquitination of Substrates and Hsp70. Nature, *440:* 551-555, 2006

Evans C. G. *et al.,* Heat Shock Protein 70 (Hsp70) as an Emerging Drug Target. J Med Chem., *53:* 4585-4602, 2010

Lanneau D. *et al.,* Heat Shock Proteins: Cell Protection through Protein Triage. The Scientific World Journal, *10:* 1543-1552, 2010

Reviewed and approved by site quality representative.