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Produktinformation

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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
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Description

The HSP70 Assay Kit is a chemiluminescence assay kit designed to measure HSP70 adenosine triphosphate (ATP) hydrolysis activity for screening and profiling applications using ADP-Glo® Assay as a detection reagent. The HSP70 Assay Kit comes in a convenient 96-well format, with enough purified HSP40 and HSP70 proteins, ATP, and HSP70 assay buffer for 100 enzyme reactions.

Background

HSP70 (Heat Shock 70kDa protein) family members are protein chaperones that play an essential role in cellular protein metabolism by acting as polypeptide-binding and release factors. These chaperones ensure the folding of newly synthesized proteins, the formation of dissociation of protein complexes, and protect against stress. HSP40 co-chaperone proteins regulate complex formation between HSP70 and client proteins. Specifically, HSP40 proteins regulate the ATP-dependent binding of HSP70 to polypeptides through their J-domain. The J-domain of HSP40 is thought to interact with HSP70 at an acidic groove located in the ATPase domain. J-domain–HSP70 complex formation plays a critical role in the regulation of HSP70 ATPase activity.

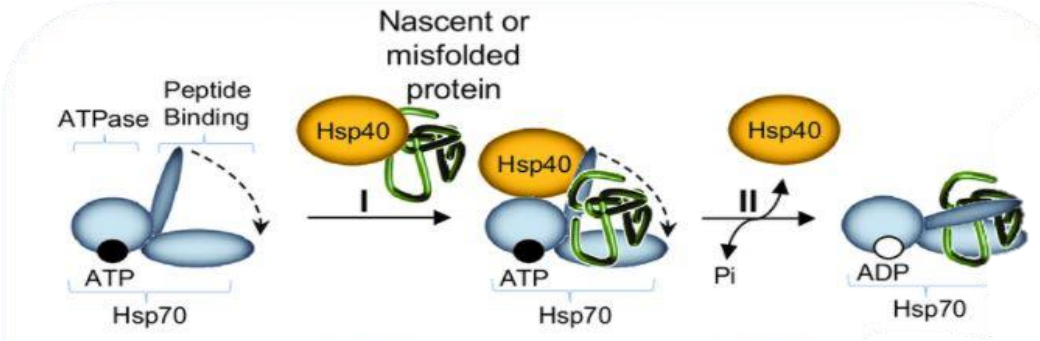

Illustration of the HSP70 machinery reaction cycle. (I) HSP40 mediates the delivery of nascent or misfolded proteins to ATP-bound HSP70; (II) Hydrolysis of ATP to ADP, accelerated by HSP40, results in HSP70 conformational change.

Applications

1. Study enzyme kinetics.
2. Screen small molecular inhibitors for drug discovery and high throughput (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
50287	HSP70*	18 µg	-80°C
78415	HSP70 Buffer	1.5 ml	-20°C
79686	ATP (500 µM)	50 µl	-20°C
50285	HSP40*	10 µg	-80°C
79696	96-well plate, white	1	Room Temp

**The concentrations of HSP40 and HSP70 are lot-specific and will be indicated on the tubes containing the proteins.*

BPS Bioscience
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

Materials Required but Not Supplied

Name	Catalog #
ADP-Glo® Assay	Promega, #V6930
Dithiothreitol (DTT; 1 M)	
Microplate reader capable of reading luminescence	
Adjustable micropipettor and sterile tips	
30°C incubator	

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

All samples and controls should be tested in duplicate.

1. Thaw the stock HSP70 buffer and ATP on ice.

   Add fresh DTT (1 M) to stock HSP70 buffer to make a 20 mM DTT concentration (e.g., for one plate add 30 µl of 1 M DTT to 1.5 ml of stock HSP70 Buffer). Use DTT-containing HSP70 Buffer all through the protocol.

   Note: Discard any remaining HSP70 assay buffer containing DTT after use.

2. Prepare the Master Mix (10 µl per well): N wells x (3 µl of stock HSP70 Buffer containing DTT + 7 µl of water). Add 10 µl to every well.

3. Prepare 3 ml of 1x HSP70 Buffer by mixing 600 µl of stock HSP70 Buffer containing DTT with 2.4 ml of water. Note that 3 ml of 1x HSP70 Buffer is sufficient for 100 reactions.

4. Prepare the Test Inhibitor (2.5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

   a) If the Test Inhibitor is water-soluble, prepare serial dilutions in the 1x HSP70 Buffer, 10-fold more concentrated than the desired final concentrations. For the positive and negative controls, use 1x HSP70 Buffer only (Diluent Solution).

b) If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in 1x HSP70 Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x HSP70 Buffer to keep the concentration of DMSO constant. For the "Positive Control" and "Blank", prepare 10% DMSO in 1x HSP70 Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

The final concentration of DMSO should not exceed 1%.

5. Thaw HSP40 on ice. Briefly spin the tube containing the HSP40 protein to recover the full content of the tube. Dilute HSP40 to 20 ng/µl with 1x HSP70 Buffer (5 µl/well).

   Notes:
   The concentration of protein is lot-specific and is indicated on the tube. Verify the initial concentration and dilute accordingly.

   HSP40 is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Although we do not recommend it, if not using all the wells of the assay at once, calculate the amount required for the assay, dilute only the amount sufficient for the assay and aliquot the remaining undiluted HSP40. Store single use aliquots at -80°C.

   Do not re-use thawed aliquots and do not re-use the diluted HSP40.

6. Add 5 µl of diluted HSP40 (20 ng/µl) to all wells (the final protein amount will be 100 ng per well).

   To the wells designated as "Blank", add 5 µl of 1x HSP70 Buffer.

7. Thaw HSP70 on ice. Briefly spin the tube containing the HSP70 to recover the full content of the tube. Dilute HSP70 to 36 ng/µl with 1x HSP70 Buffer (5 µl/well).

   Notes:
   The concentration of protein is lot-specific and is indicated on the tube. Verify the initial concentration and dilute accordingly.

   HSP70 is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Although we do not recommend it, if not using all the wells of the assay at once, calculate the amount required for the assay, dilute only the amount sufficient for the assay and aliquot the remaining undiluted HSP70. Store single use aliquots at -80°C.

   Do not re-use thawed aliquots and do not re-use the diluted HSP70.

8. Add 5 µl of diluted HSP70 (36 ng/µl) to the wells designated "Positive Control" and "Test Inhibitor". The final amount of HSP70 will be 180 ng per well.

Preincubate the mixture for **30 minutes** in room temperature with slow shaking. Please ensure that the mixture covers the entire surface of the plate to obtain consistent results. For example, you may want to tap it carefully or put it on agitator for 2-3 minutes (avoid splashing).

9. After 30 minutes, dilute ATP in distilled water at a final concentration of 50 µM (i.e. make a 10-fold dilution using stock ATP at 500 µM). Initiate the reaction by adding 2.5 µl of diluted ATP to all wells. Mix it thoroughly by placing it for 2-3 minutes on the shaker then incubate at 30°C for 60 minutes.

Component	**Blank**	**Positive Control**	**Test Inhibitor**
Master Mix	10 µl	10 µl	10 µl
Test Inhibitor	-	-	2.5 µl
Diluent Solution (No inhibitor)	2.5 µl	2.5 µl	-
HSP40 (20 ng/µl)	5 µl	5 µl	5 µl
1x HSP70 buffer	5 µl	-	-
HSP70 (36 ng/µl)	-	5 µl	5 µl
ATP (50 µM)	2.5 µl	2.5 µl	2.5 µl
Total	25 µl	25 µl	25 µl

10. Thaw the ADP-Glo reagent.

    After the 60 minutes reaction, add 25 µl of ADP-Glo reagent to each well. Cover the plate with aluminum foil and incubate the plate at room temperature for 60 minutes.

11. Thaw the Detection reagent.

    After the 60 minutes incubation, add 50 µl of Detection reagent to each well. Cover the plate with aluminum foil and incubate the plate at room temperature for another 45 minutes.

    Immediately read the plate in a luminometer or microtiter-plate capable of reading chemiluminescence. The “Blank” value is subtracted from all readings.

Example Results

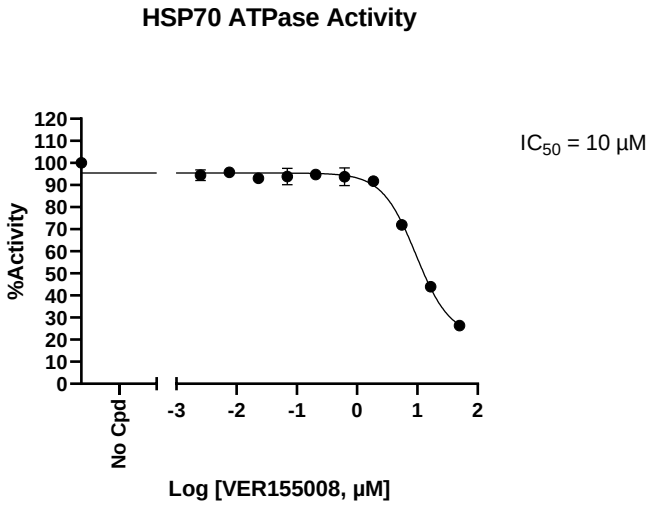

Figure 1: Inhibition of HSP70 ATPase activity by VER155008. HSP70 activity was measured in the presence of increasing concentrations of VER155008 using the HSP70 assay kit (BPS Bioscience #78414) at a final concentration of ATP of 5 µM. Results are expressed as percent of positive control (the positive control "no inhibitor" value was set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

References

1. Fan CY, e*t al.* Mechanisms for regulation of Hsp70 function by Hsp40. *Cell stress & chaperones*, 2003; **8(4):** 309–316.
2. Massey AJ, *et al.* A novel, small molecule inhibitor of Hsc70/Hsp70 potentiates Hsp90 inhibitor induced apoptosis in HCT116 colon carcinoma cells. *Cancer Chemother Pharmacol*. **2010**; 66(3): 535-45.
3. Shiber A, Ravid T. Chaperoning proteins for destruction: diverse roles of Hsp70 chaperones and their co-chaperones in targeting misfolded proteins to the proteasome. *Biomolecules*. 2014; **4(3)**: 704-24.

Related Products

Products	*Catalog #*	*Size*
HSP70, His-Tag	50287	200 µg
HSP40, His-Tag	50285	10 µg
HSP70 (Q435A), His-Tag	50288	100 µg