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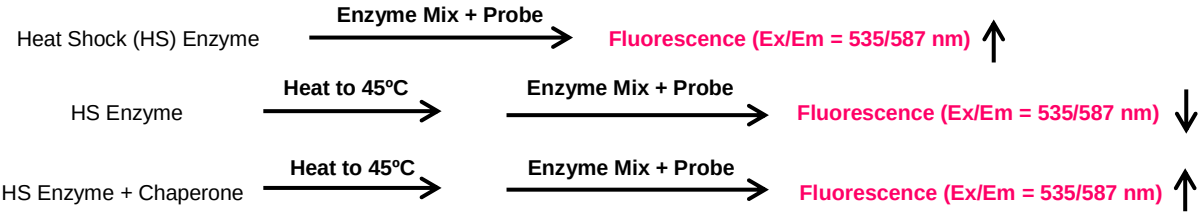
Heat Shock Protein Assay Kit (Fluorometric)

10/19

(Catalog # K2024-100; 100 assays; Store at - 80ºC)

I. Introduction:

Thermal stress or elevated temperatures cause proteins to denature thereby resulting in the loss of function leading to disruption in protein homeostasis. All organisms express a class of proteins known as **Heat Shock Proteins (HSPs)** to circumvent this problem by stabilizing the protein structure during stressful conditions. The name "Heat Shock Protein" comes from the original discovery that the abundance of these proteins is elevated during heat stress. Although HSPs are constitutively expressed and are involved in protein stabilization by ensuring the correct folding of newly synthesized proteins, their abundance is increased during elevated temperatures, high salt concentration, or altered pH. Under such stressful conditions, HPSs bind to proteins thereby providing protection from misfolding and aggregation. HSPs are named according to their molecular weights. Some of the most widely studied HSPs are HSP60, HSP70 and HSP90 with a molecular mass of 60, 70 and 90 kDa respectively. HSP90 is known to stabilize tumor specific proteins required for tumor growth and thus its inhibitors are being investigated as potential therapeutic targets. **BioVision's Heat Shock Protein Assay Kit** is a simple plate based assay that measures the activity of an enzyme in its native state and after being subjected to heat stress at 45ºC in the presence and absence of an HSP. Protective effect of the HSP is expressed as the % activity of the enzyme heated in the presence of HSP as compared to the activity of the native enzyme.



II. Applications:

Quantification of enzyme activity preserved by heat shock proteins

III. Sample Type:

- Isolated heat shock proteins
- Isolated proteins with chaperone like activity

IV. Kit Contents:

Components	K2024-100	Cap Code	Part Number
HS Assay Buffer	25 ml	WM	K2024-100-1
HS Enzyme	1 vial	Orange	K2024-100-2
HS Developer	1 vial	Red	K2024-100-3
HS Probe	0.4 ml	Blue	K2024-100-4
HSP70	1 vial	Yellow	K2024-100-5

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer
- Distilled water

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -80°C, protected from light. All kit components except HSP70 Positive Control may be stored at -20°C if desired. HSP70 must be stored at -80°C. Briefly centrifuge all small vials before opening. Read the entire protocol before performing the assay. Components are stable for at least three months.

- **HS Assay Buffer:** Warm to room temperature (RT) before use.
- **HS Enzyme:** Reconstitute the vial in 100 µl HS assay buffer to prepare the reconstituted HS Enzyme. Aliquot and store at -20ºC. Avoid multiple freeze thaw cycles.
- **HS Developer:** Reconstitute the vial in 220 µl HS assay buffer. Aliquot and store at -20ºC in the dark. Thaw on ice before use.
- **HS Probe:** Provided as a solution in DMSO. Divide into aliquots and store at -20°C, protected from light. Prior to use, warm to RT.
- **HSP70:** Reconstitute the vial with 25 µl dH_2O. Aliquot and store at -80ºC. Avoid multiple freeze thaw cycles.

VII. HSP Assay Protocol:

1. **Test Protein Preparation:** Prepare diluted HS Enzyme by diluting the reconstituted HS Enzyme at 1:50 dilution with distilled water**.** Test proteins should be prepared at a starting concentration of 0.5-2 mg/ml. Prepare the following wells in a 96 well white plate:

	Sample	HSP Positive Control (PC)	Denatured Enzyme Control (DE)
HS Assay Buffer	73-76 µl	76 µl	78 µl
Sample Protein	2-5 µl	-	-
HSP70	-	2 µl	-
Diluted HS Enzyme	2 µl	2 µl	2 µl

Incubate the plate at 45ºC for 60 min. At the end of 60 min incubation, cool the plate to ambient temperature and prepare two additional wells labeled as **"Native Enzyme Control (NE)"** and **"Assay Background Control (BC)"** respectively. For NE well, add 2 µl of the diluted HS Enzyme and bring up the volume to 80 µl using HS Assay Buffer. For BC well, add 80 µl of HS Assay Buffer.

2. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total of 20 µl Reaction Mix:

	Reaction Mix
HS Assay Buffer	16 µl
HS Developer	2 µl
HS Probe	2 µl

Mix well and add 20 µl of Reaction Mix to all wells including **Sample**, **PC**, **DE**, **NE** and **BC** wells.

Notes:

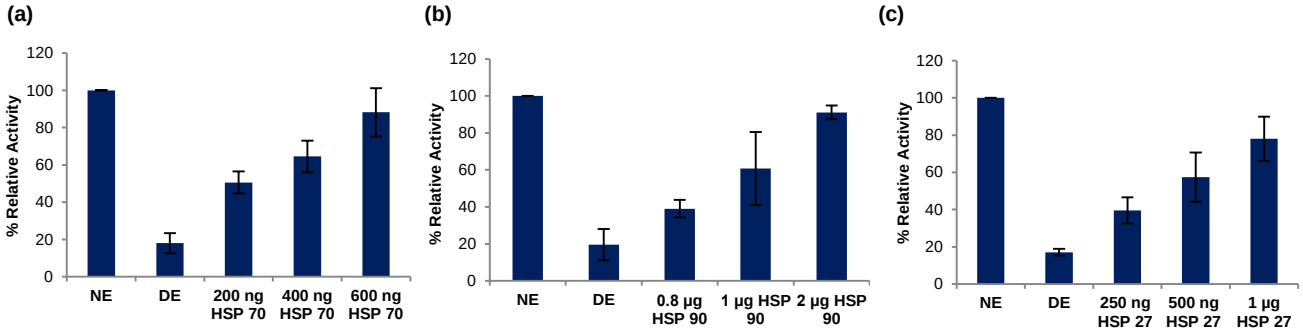
a) Have the microplate reader ready at Ex/Em = 535/587 nm in kinetic mode at RT set to record fluorescence every 30 sec.

b) Prepare Reaction Mix immediately before adding to the wells.

3. Measurement: Immediately start recording fluorescence at 30 sec intervals for 15-30 min at RT.

4. Calculation: Subtract **BC** readings from **PC, NE, DE and Sample** wells**.** Obtain ΔRFU for NE, DE and Sample wells by subtracting RFU at time t_1 from RFU at time t_2, such that t_2 and t_1 is within a linear range of the assay. Calculate slope for all Samples by dividing ΔRFU by time Δt ($t_2 - t_1$). Obtain % Relative Activity by using the calculations as shown below:

$$\textbf{\% Relative Activity} = \frac{[slope\ (NE)]\ or\ [slope\ (DE)]\ or\ [slope\ (test\ protein)]}{slope\ (NE)}\ X\ 100$$



Figures: % Relative Activity of enzyme subjected to heat stress in the presence of (a) HSP70, (b) HSP90 and (c) HSP27 respectively.

VIII. Related Products:

Heat Shock Protein 90 (Hsp90 beta) NT-His Tag, human recombinant (4858H)
Heat Shock Protein 90 (Hsp90), human recombinant (4858)
Heat Shock Protein 70, human recombinant (4859)
Heat Shock Protein 65, mycobacterium recombinant (4856)
Heat Shock Protein 27, human recombinant (4853)
Heat Shock Protein 22, human recombinant (4850)
Heat Shock Protein 20, human recombinant (4847)

FOR RESEARCH USE ONLY! Not to be used on humans.