ab284527 – Heat Shock Protein Assay Kit (Fluorometric)

For the measurement of Heat Shock Protein.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284527

Storage and Stability

On receipt entire assay kit should be stored at -80°C, protected from light. Upon opening, use kit within 3 months.

Materials Supplied

Item	Quantity	Storage Condition
HS Assay Buffer	25 mL	-80°C
HS Developer	1 vial	-80°C
HS Enzyme	1 vial	-20°C
HS Probe	0.4 mL	-80°C
HSP70	1 vial	-80°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

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 -20°C. Avoid multiple freeze thaw cycles.

HS Developer: Reconstitute the vial in 220 µl HS assay buffer. Aliquot and store at -20°C 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 dH2O. Aliquot and store at -80°C. Avoid multiple freeze thaw cycles.

Assay Protocol

Test Protein preparation:

1. 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

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2. Incubate the plate at 45°C 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.

Reaction Mix:

1. Mix enough reagents for the number of assays to be performed, immediately before adding to the plate.
2. Prepare just enough for the number of reactions being run immediately.
3. Keeps the vial containing the reaction mix on ice while adding it to the well of the 96 well plate. For each well, prepare 20 µl Mix containing:

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

4. Mix well and add 20 µl of the Reaction Mix to wells containing sample, PC, DE, NE, BC.

Δ Note: *Have the plate reader ready at Ex/Em= 535/587 nm on kinetic mode at RT set to record fluorescence every 30 seconds.*

Measurement

Start recording fluorescence at Ex/Em= 380/460 nm after adding the substrate at 30 second intervals for 15-30 minutes at RT

Calculation:

1. Subtract BC readings from PC, NE, DE and Sample wells. Obtain ΔRFU for NE, DE and Sample wells by subtracting RFU at time t1 from RFU at time t2, such that t2 and t1 is within a linear range of the assay.
2. Calculate slope for all Samples by dividing ΔRFU by time Δt (t2 – t1). Obtain % Relative Activity by using the calculations as shown below:

$$\% \text{ Relative activity} = \frac{[slope\ (NE)]or\ [clope(DE)]or\ [slope(test\ protein)]}{slope\ (NE)} \times 100$$

Technical Support

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