ab308155 – HSP90a Inhibitor Screening Kit (Fluorometric)

For screening or characterizing HSP90a inhibitors
For research use only - not intended for diagnostic use.

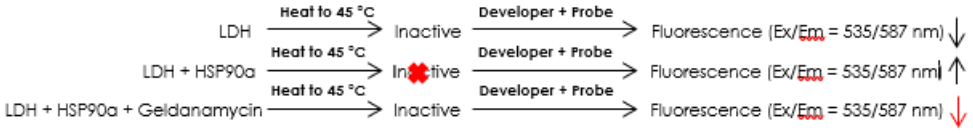
For overview, typical data and additional information please visit:

http://www.abcam.com/ab308155

Storage and Stability
The entire Assay kit may be stored at -80 °C and protected from light.

Introduction:

Heat shock protein (HSP) 90 is an ATP-dependent molecular chaperone that assists other proteins to fold properly, stabilizes proteins against heat stress, and assists in protein degradation. It has two isoforms (HSP90a and HSP90β) and both are abundantly expressed in the cytoplasm. Heat stress can cause proteins to denature thereby resulting in the loss of protein function. However, HSP90 can bind to the proteins during heat stress and protect them from misfolding and aggregation. Since HSP90 stabilizes various proteins including cancer-related proteins, cancer-associated protein kinases, transcription factors etc. that are required for the survival of cancer cells, HSP90 is considered as a potential target for cancer therapeutics. HSP90 inhibitors can target either the N-terminal ATP binding site or the C-terminal site of HSP90. Additionally, a number of HSP90 inhibitors are in clinical trials. **The HSP90a Inhibitor Screening Kit** is a 96-well fluorometric assay that can be used to screen and characterize the potential inhibitors of HSP90a. The assay is based on measuring the HSP90-dependent refolding of thermally denatured Lactate Dehydrogenase (LDH) in the presence and absence of potential HSP90a inhibitors. In the presence of potential HSP90a inhibitors, refolding of thermally denatured LDH will be arrested resulting in reduced LDH activity, which is measured by fluorescence at Ex/Em = 535/587 nm. Geldanamycin is included in the kit as a positive control. The assay is simple, rapid and provides a reliable test for screening HSP90a inhibitors.



Materials Supplied

Item	Quantity
HSP Assay Buffer	25 mL
LDH	20 µl
LDH Developer	1 vial
LDH Probe	400 µl
ATP	1 vial
HSP90a	20 µl
Geldanamycin	20 µl

Materials Required, Not Supplied

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

- dH_2O
- DMSO (≥ 99.9%)
- 96-well white plate with flat bottom
- Multi-well spectrophotometer
- PCR tubes or micro-centrifuge tubes (0.6 ml)

Reagent Preparation

- Store the kit at -80 °C, protected from light.
- All the kit components except HSP90a may be stored at -20 °C if desired.
- **HSP90a must be stored at -80 °C.**
- Briefly centrifuge all small vials prior to opening.
- Read the entire protocol before performing the assay

HSP Assay Buffer: Store at -20 °C or 4 °C .Warm to room temperature (RT) before use.

LDH: Reconstitute the vial in 100 µl HSP assay buffer to prepare the LDH stock. Divide into aliquots and store at -20 °C. Avoid multiple freeze thaw cycles.

LDH Developer: Reconstitute the vial in 220 µl HSP assay buffer. Divide into aliquots and store at -20 °C in the dark. Thaw on ice before use.

LDH Probe (in DMSO): Ready to use. Thaw at RT before use to melt the DMSO. Store at -20 °C.

ATP: Reconstitute the vial with 220 µl dH_2O to generate ATP stock solution. Divide into aliquots and store at -20°C. Keep on ice while in use.

HSP90a: Ready to use. Divide into aliquots and Store at -80 °C. Avoid multiple freeze-thaw cycles. Keep on ice while in use. Note: Please keep on dry ice, if not using immediately.

Geldanamycin (in DMSO): Ready to use. Bring to RT before use. Store at -20 °C.

Sample Preparation

Screening Compounds, Inhibitor Control & Background Controls preparations:
Prepare 2% (v/v) DMSO. (i.e. add 2 µl of DMSO (≥ 99.9%) with 98 µl dH_2O).
Sample: Dissolve Test sample(s) in an appropriate solvent to prepare the stock solution. Further dilute the stocks to working solution in lower concentration of the solvent.
HSP90a: Prepare 1:6 dilution of HSP90a in dH_2O (i.e. mix 2 µl of HSP90a stock solution with 10 µl dH_2O).
Inhibitor: Prepare 1:10 dilution of Geldanamycin stock in 2% DMSO (i.e. mix 2 µl of Geldanamycin stock with 18 µl of 2% DMSO).

Prepare the Experimental Conditions as shown below in PCR tubes or in 0.6 ml microtubes:

	Sample [S]	Enzyme [E]	Inhibitor [I]	Sample Control [SC]	Enzyme Control [EC]	Inhibitor Control [IC]
Diluted Geldamycin	–	–	2 µl	–	–	2 µl
Sample	2 µl	–	–	2 µl	–	–
2% DMSO or Final solvent conc.* of Sample*	–	2 µl	–	–	2 µl	–
Diluted HSP20a	2 µl	2 µl	2 µl	–	–	–
dH_2O	–	–	–	2 µl	2 µl	2 µl
HSP Assay Buffer	36 µl	36 µl	36 µl	36 µl	36 µl	36 µl
Total Volume	40 µl	40 µl	40 µl	40 µl	40 µl	40 µl

Mix well and spin down. Incubate the individual PCR tubes or the 0.6 ml tubes at 45 °C for 20-25 min in a thermal shaker at 300-500 rpm. Ensure the cap is securely tightened and protected from light.

*Various organic solvents may affect the HSP90a activity (see Fig b). If samples are prepared in organic solvent, we recommend dissolving the test samples to higher concentrations in organic solvent. Further dilute the test samples(s) to a lower concentration of organic solvent (such as 2% DMSO; 2% DMF) or dH_2O to minimize the effect of organic solvent(s). Prepare parallel well(s) with the same final concentration (conc.) of organic solvent on HSP90a [E] to test the effect of the solvent on HSP90a activity.

LDH Preparation:
Prepare 1:100 dilution of LDH with dH_2O (i.e. mix 2 µl of LDH stock with 198 µl dH_2O) and mix well. Prepare 1:200 dilution of ATP with dH_2O (i.e. mix 2 µl of ATP stock with 398 µl dH_2O). Further prepare 1:250 dilution of ATP with dH_2O (i.e. mix 2 µl of diluted ATP with 498 µl of dH_2O). Mix enough reagents for the number of assays to be performed. For each tube, prepare a total of 40 µl LDH Mix:

	LDH Mix
HSP Assay Buffer	36 µl
Diluted ATP	2 µl
Diluted LDH	2 µl

Add 40 µl of LDH Mix to each tube from step VI.1 designated as Sample[S], Enzyme [E], Inhibitor [I] and Controls ([SC], [EC] and [IC]). Spin down the tubes to mix and incubate at 45 °C for 1 hr in a thermal shaker at 300-500 rpm. Note: Ensure the cap is securely tightened and the tubes are protected from light. After the end of 1 hr incubation, put tubes at RT for 10-20 min to cool down, avoid light. Transfer 80 µl of each experimental condition into the desired well(s) of a white, flat-bottom 96-well plate. Note: Prepare LDH Mix immediately before adding to the tubes.

Assay Procedure

1. **Reaction Mix Preparation**: For each well, prepare 20 µl Reaction Mix containing:

	Reaction Mix
HSP Assay Buffer	16 µl
LDH Developer	2 µl
LDH Probe	2 µl

   Mix well and add 20 µl of Reaction Mix to all wells including Sample(s), Inhibitor, Enzyme, Sample Control, Enzyme Control and Inhibitor Control. **Note:** Prepare Reaction Mix immediately before adding to the wells.

2. **Measurement**: Measure the fluorescence immediately at 535/587 nm for 60-90 min at RT. Choose any two time points (t_1 & t_2) in the linear range of the curve and obtain the corresponding RFU for all Samples [S], Enzyme [E], Sample Control [SC] and Enzyme Control [EC].
   Note: It is normal to observe a lag phase in the first 20 min. Linear range is usually observed after 40 min of reaction.
3. **Calculation**: Calculate the slope for all Samples [S], Enzyme [E], Sample Control [SC] and Enzyme Control [EC] by dividing the net ΔRFU ($R_{t2} - R_{t1}$) values over reaction time Δt ($t_2 - t_1$).

$$\% \text{ Inhibition} = \frac{\text{Slope (E–EC)} - \text{Slope (S–SC)}}{\text{Slope (E–EC)}} \times 100$$

$$\% \text{ Relative Activity} = \frac{\text{Slope [S–SC]}}{\text{Slope [E–EC]}} \times 100$$

For technical support contact information, visit: www.abcam.com/contactus