

Hsp90 β (N-Terminal) Assay Kit**Description**

Hsp90 β (heat shock protein 90) is a molecular chaperone with essential functions in maintaining transformation. Inhibition of Hsp90 β function has been shown to play a role in tumorigenesis and disease progression. The Hsp90 β Assay Kit is designed for the identification of Hsp90 β inhibitors using fluorescence polarization. The assay is based on the competition of fluorescently labeled geldanamycin, an HSP90 inhibitor, for binding to purified recombinant Hsp90 β .

The Hsp90 β Assay Kit comes in a convenient 384-well format, with enough purified Hsp90 β enzyme, FITC-labeled geldanamycin, and Hsp90 β assay buffer for 400 enzyme reactions. The key to the Hsp90 β Assay Kit is the fluorescently labeled geldanamycin. Only one simple step on a microtiter plate is required for Hsp90 β reactions. The FITC-labeled geldanamycin is incubated with a sample containing Hsp90 β enzyme to produce a change in fluorescent polarization. The FP signal is measured using a fluorescent microplate reader *capable of measuring fluorescence polarization*.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage	
50292	HSP90 β *	140 μ g	-80°C	Avoid multiple freeze/thaw cycles
50312	FITC-labeled geldanamycin (2.5 μ M)	2 x 30 μ l	-80°C	
50311	5x Hsp90 assay buffer 1	2 x 4 ml	-20°C	
	Black, low binding microtiter plate	1	Room Temp	

*The initial concentration of enzyme is lot-specific and will be indicated on the tube containing the protein.

Materials Required but Not Supplied

Name	Catalog #
40 M DTT	
2 mg/ml BSA (bovine serum albumin)	

Storage Conditions

This assay kit will perform optimally for up to 12 months from date of receipt when the materials are stored as directed. Avoid multiple freeze/ thaw cycles!

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**Reagent Preparation**

1. Thaw **FITC-labeled geldanamycin** on ice.
 - a. Briefly spin tube containing FITC-labeled geldanamycin to recover full content of the tube.
 - b. Aliquot into single-use aliquots. Store remaining FITC-labeled geldanamycin in aliquots at -80°C immediately.
2. Thaw **Hsp90 β** on ice.
 - a. Briefly spin tube containing Hsp90 β to recover full content of the tube.
 - b. Aliquot Hsp90 β into single use aliquots. Store remaining Hsp90 β in aliquots at -80°C immediately.



*Note: **FITC-labeled geldanamycin** and **Hsp90 β** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

Reaction Setup

All samples and controls should be tested in duplicate.

1. Dilute FITC-labeled geldanamycin (2.5 μM stock) 25-fold with 1x Hsp90 assay buffer to make a 100 nM solution.

Note: Make only sufficient quantity needed for the assay; store remaining 2.5 μM stock solution in aliquots at -80°C .

2. Dilute Hsp90 β in 1x Hsp90 assay buffer to 35 ng/ μl (350 ng/reaction)*. Aliquot any remaining enzyme and store undiluted at -80°C . Keep diluted enzyme on ice. Discard any remaining diluted enzyme after use.

**Note: Optimal enzyme concentration may vary with the specific activity of the enzyme.*

3. Prepare the master mixture: N wells x (7.5 μl **5x Hsp90 assay buffer 1** + 2.5 μl **40 mM DTT** + 2.5 μl **2 mg/ml BSA** + 20 μl **H₂O**). Add 32.5 μl of master mixture to all wells.

Component	Blank	Enzyme Positive Control	Enzyme Negative Control	Test Inhibitor
5x Hsp90 assay buffer 1	7.5 μl	7.5 μl	7.5 μl	7.5 μl
40 mM DTT	2.5 μl	2.5 μl	2.5 μl	2.5 μl
2 mg/ml BSA	2.5 μl	2.5 μl	2.5 μl	2.5 μl
H ₂ O	20 μl	20 μl	20 μl	20 μl
FITC-Labeled geldanamycin (100 nM)	–	2.5 μl	2.5 μl	2.5 μl
Inhibitor	–	–	–	5 μl
Inhibitor Buffer (no inhibitor)	5 μl	5 μl	5 μl	–
1x HSP90 assay buffer	12.5 μl	–	10 μl	–
Hsp90 β (35 ng/ μl)	–	10 μl	–	10 μl
Total	50 μl	50 μl	50 μl	50 μl

4. Add 2.5 μl of diluted **FITC-labeled geldanamycin** (100 nM) to each well designated “Enzyme Positive Control”, “Enzyme Negative Control”, and “Test Inhibitor.”
5. Add 5 μl of **Inhibitor** to each well designated “Test Inhibitor.” For the, “Blank”, “Enzyme Positive Control” and “Enzyme Negative Control”, add 5 μl of the same solution without Inhibitor (**Inhibitor Buffer**).
6. Add 10 μl of **1x HSP90 assay buffer** to the well designated “Enzyme Negative Control”. Add 12.5 μl **1x Hsp90 assay buffer** to the wells designated “Blank”.
7. Initiate reaction by adding 10 μl of **diluted Hsp90β** (35 ng/μl), prepared as described above, to each well designated “Enzyme Positive Control” and “Test Inhibitor.”
8. Incubate at room temperature for 2-3 hours with slow shaking.

Reaction detection and reading results

Read fluorescent polarization of the sample in a microtiter-plate reader capable of excitation at wavelengths ranging from 475-495 nm and detection of emitted light ranging from 518-538 nm. Blank value is subtracted from all other values.

Calculating Results

Definition of Fluorescence Polarization:

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

Where I_{\parallel} = Intensity with polarizers parallel and I_{\perp} = Intensity with polarizers perpendicular. Most instruments display fluorescence polarization in units of mP.

$$mP = \left(\frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} \right) \times 1000$$

The equation above assumes that light is transmitted equally well through both parallel and perpendicular oriented polarizers. In practice, this is generally not true and a correction must be made to measure the absolute polarization state of the molecule. This correction factor is called the "G Factor".

$$mP = \left(\frac{I_{\parallel} - G(I_{\perp})}{I_{\parallel} + G(I_{\perp})} \right) \times 1000 \quad \text{OR} \quad mP = \left(\frac{G(I_{\parallel}) - I_{\perp}}{G(I_{\parallel}) + I_{\perp}} \right) \times 1000$$

The G-factor is instrument-dependent and may vary slightly depending upon instrument and conditions. Please check the manual of your instrument to obtain the information about the establishment of the G-factor.

Example Results

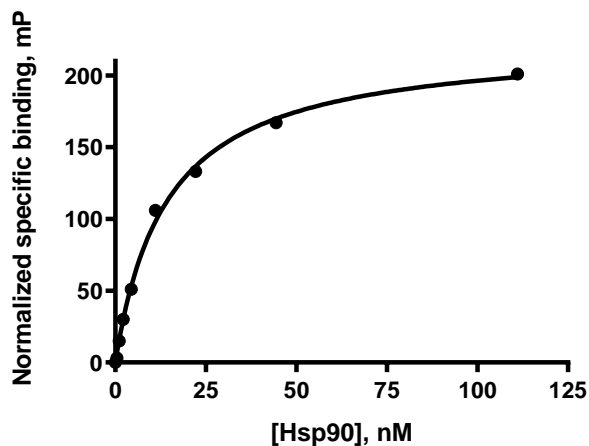


Figure 1: Binding of FITC-geldanamycin to HSP90 β .

Binding was measured using the Hsp90 β Assay Kit (BPS Bioscience #50299). Fluorescence was measured at λ_{ex} 485nm, λ_{em} 530 nm using a Bio-Tek fluorescent microplate reader.

References

1. Kim J, et al., *Biomol. Screening* 2004; 9(5): 375-381.
2. Howes R, et al., *Anal. Biochem.* 2006; 350:202-213.

Related Products

Product	Catalog #	Size
HSP90 α , His-tag Recombinant	50290	200 μ g
HSP90 β , His-tag Recombinant	50292	200 μ g
AHA1, His-tag Recombinant	50291	200 μ g
Geldanamycin	27008	5 mg
MS-275 (Entinostat)	27011	25 mg
Hsp90 α N-Terminal Domain Assay Kit	50293	96 rxns
Hsp90 α (N-terminal) Assay Kit	50298	384 rxns
HSP90 α C-Terminal Domain TR-FRET Assay Kit	50289	96 rxns
HSP90 α (C-Terminal Domain) TR-FRET Kit	50261	384 rxns
HSP90 α (C-Terminal) Inhibitor Screening Assay Kit	50317	384 rxns
Hsp90 β N-Terminal Domain Assay Kit	50294	96 rxns
HSP90 β (C-Terminal Domain) TR-FRET Kit	50262	384 rxns
HSP90 β (C-terminal) Inhibitor Screening Kit	50314	384 rxns