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**Data Sheet**  
***Hsp90 $\beta$*  Assay Kit**  
Catalog # 50299  
Size: 384 reactions

**DESCRIPTION:** Hsp90 $\beta$  is a molecular chaperone with essential functions in maintaining transformation. Inhibition of Hsp90 $\beta$  function has been shown to play a role in tumorigenesis and disease progression. The *Hsp90 $\beta$  Assay Kit* is designed for identification of Hsp90 $\beta$  inhibitors using fluorescence polarization. The assay is based on the competition of fluorescently labeled geldanamycin, an HSP90 inhibitor, for binding to purified recombinant Hsp90 $\beta$ .

The *Hsp90 $\beta$  Assay Kit* comes in a convenient 384-well format, with enough purified Hsp90 $\beta$  enzyme, FITC-labeled geldanamycin, and Hsp90 $\beta$  assay buffer for 400 enzyme reactions. The key to the *Hsp90 $\beta$  Assay Kit* is the fluorescently labeled geldanamycin. Using this kit, only one simple step on a microtiter plate is required for Hsp90 $\beta$  reactions. The FITC-labeled geldanamycin is incubated with a sample containing Hsp90 $\beta$  enzyme to produce a change in fluorescent polarization that can then be measured using a fluorescence reader.

**COMPONENTS:**

Catalog #	Component	Amount	Storage	
50292	Hsp90 $\beta$ recombinant enzyme	140 $\mu$ g	-80°C	<b>Avoid freeze/ thaw cycles!</b>
	FITC-labeled geldanamycin (2.5 $\mu$ M)	2 x 30 $\mu$ l	-80°C	
50311	5x Hsp90 assay buffer 1	2 x 4 ml	-20°C	
	Black, low binding microtiter plate	1	Room temp.	

**MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:**

40 mM DTT  
2 mg/ml BSA (bovine serum albumin)

**APPLICATIONS:** Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

**STABILITY:** Up to 1 year when stored as recommended.

**REFERENCES:**

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

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## ASSAY PROTOCOL:

### Immediately prior to assay:

- 1) Thaw **FITC-labeled geldanamycin** on ice. Upon first thaw, briefly spin tube containing FITC-labeled geldanamycin to recover full content of the tube. Aliquot into single use aliquots. Store remaining FITC-labeled geldanamycin in aliquots at  $-80^{\circ}\text{C}$  immediately. *Note: FITC-labeled geldanamycin is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*
- 2) Thaw **Hsp90 $\beta$**  on ice. Upon first thaw, briefly spin tube containing Hsp90 $\beta$  to recover full content of the tube. Aliquot Hsp90 $\beta$  into single use aliquots. Store remaining Hsp90 $\beta$  in aliquots at  $-80^{\circ}\text{C}$  immediately. *Note: Hsp90 $\beta$  is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

### Step 1:

#### **All samples and controls should be tested in duplicate.**

- 1) Dilute FITC-labeled geldanamycin (2.5  $\mu\text{M}$  stock) 25-fold with 1x Hsp90 assay buffer to make a 100 nM solution. (Make only sufficient quantity needed for the assay; store remaining 2.5  $\mu\text{M}$  stock solution in aliquots at  $-80^{\circ}\text{C}$ .)
- 2) Dilute Hsp90 $\beta$  in 1x Hsp90 assay buffer to 35 ng/ $\mu\text{l}$  (350 ng/reaction)\*. Aliquot any remaining enzyme and store undiluted at  $-70^{\circ}\text{C}$ . 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  $\mu\text{l}$  **5x Hsp90 assay buffer 1** + 2.5  $\mu\text{l}$  **40 mM DTT** + 2.5  $\mu\text{l}$  **2 mg/ml BSA** + 20  $\mu\text{l}$  **H<sub>2</sub>O**). Add 32.5  $\mu\text{l}$  of master mixture to all wells.

	Blank	Enzyme Positive Control	Enzyme Negative Control	Test Inhibitor
5x Hsp90 assay buffer 1	7.5 $\mu\text{l}$	7.5 $\mu\text{l}$	7.5 $\mu\text{l}$	7.5 $\mu\text{l}$
40 mM DTT	2.5 $\mu\text{l}$	2.5 $\mu\text{l}$	2.5 $\mu\text{l}$	2.5 $\mu\text{l}$
2 mg/ml BSA	2.5 $\mu\text{l}$	2.5 $\mu\text{l}$	2.5 $\mu\text{l}$	2.5 $\mu\text{l}$
H <sub>2</sub> O	20 $\mu\text{l}$	20 $\mu\text{l}$	20 $\mu\text{l}$	20 $\mu\text{l}$
FITC-Labeled geldanamycin (100 nM)	–	2.5 $\mu\text{l}$	2.5 $\mu\text{l}$	2.5 $\mu\text{l}$
Inhibitor	–	–	–	5 $\mu\text{l}$
Inhibitor Buffer (no inhibitor)	5 $\mu\text{l}$	5 $\mu\text{l}$	5 $\mu\text{l}$	–
1x HSP90 assay buffer	12.5 $\mu\text{l}$	–	10 $\mu\text{l}$	–
Hsp90 $\beta$ (35 ng/ $\mu\text{l}$ )	–	10 $\mu\text{l}$	–	10 $\mu\text{l}$
<b>Total</b>	<b>50 <math>\mu\text{l}</math></b>	<b>50 <math>\mu\text{l}</math></b>	<b>50 <math>\mu\text{l}</math></b>	<b>50 <math>\mu\text{l}</math></b>

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

#### Step 2:

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".

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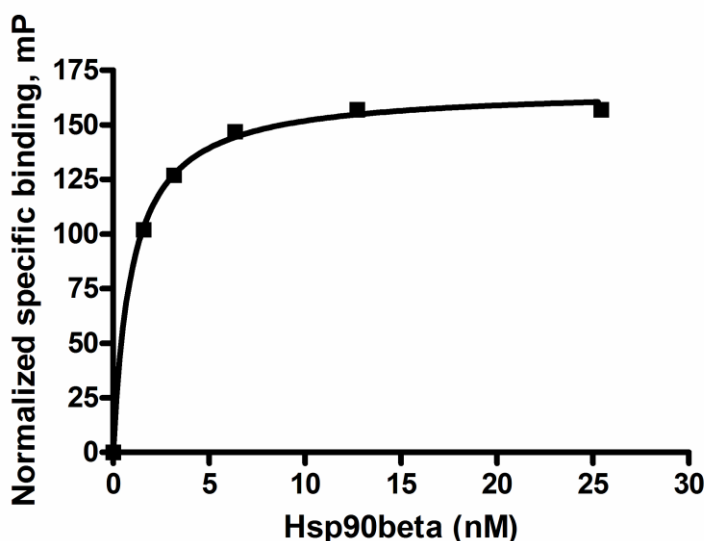


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$$mP = \left( \frac{I_{II} - G(I_1)}{I_{II} + G(I_1)} \right) \times 1000 \quad \text{OR} \quad mP = \left( \frac{G(I_{II}) - I_1}{G(I_{II}) + I_1} \right) \times 1000$$

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 OF ASSAY RESULTS:**



Binding of FITC-geldanamycin to HSP90β, measured using the Hsp90β Assay Kit, BPS Bioscience # 50299. Fluorescence was measured at λ<sub>ex</sub> 485nm, λ<sub>em</sub> 530 nm using a Bio-Tek fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at [info@bpsbioscience.com](mailto:info@bpsbioscience.com)*

**RELATED PRODUCTS:**

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
Hsp90α recombinant enzyme	50290	200 µg
Hsp90β recombinant enzyme	50292	200 µg
Aha1 recombinant enzyme	50291	200 µg
Geldanamycin inhibitor	27008	5 mg
MS-275 (Entinostat) inhibitor	27011	25 mg
Hsp90α Assay Kit (96 well)	50293	96 rxns
Hsp90α Assay Kit (384 well)	50298	384 rxns
Hsp90β Assay Kit (96 well)	50294	96 rxns

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