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Data Sheet
***Hsp90 α* Assay Kit**
Catalog # 50298
Size: 384 reactions

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

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

COMPONENTS:

Catalog #	Component	Amount	Storage	
50290	Hsp90 α recombinant enzyme	140 μ g	-80°C	Avoid 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	
79961	Low binding black microtiter 384-well 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°C immediately. *Note: FITC-labeled geldanamycin is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*
- 2) Thaw **Hsp90α** on ice. Upon first thaw, briefly spin tube containing Hsp90α to recover full content of the tube. Aliquot Hsp90α into single use aliquots. Store remaining Hsp90α in aliquots at -80°C immediately. *Note: Hsp90α 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 μM 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 μM stock solution in aliquots at -80°C.)
- 2) Dilute Hsp90α 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.

	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	-
Hsp90α (35 ng/μl)	-	10 μl	-	10 μl
Total	50 μl	50 μl	50 μl	50 μl

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- 4) Add 2.5 μ l of diluted **FITC-labeled geldanamycin** (100 nM) to each well designated "Enzyme Positive Control", "Enzyme Negative Control", and "Test Inhibitor."
- 5) Add 5 μ l of **Inhibitor** to each well designated "Test Inhibitor." For the, "Blank", "Enzyme Positive Control" and "Enzyme Negative Control", add 5 μ l of the same solution without Inhibitor (**Inhibitor Buffer**).
- 6) Add 10 μ l of **1x HSP90 assay buffer** to the wells designated "Enzyme Negative Control". Add 12.5 μ l **1x Hsp90 assay buffer** to the wells designated "Blank".
- 7) Initiate reaction by adding 10 μ l of **diluted Hsp90 α** (35 ng/ μ 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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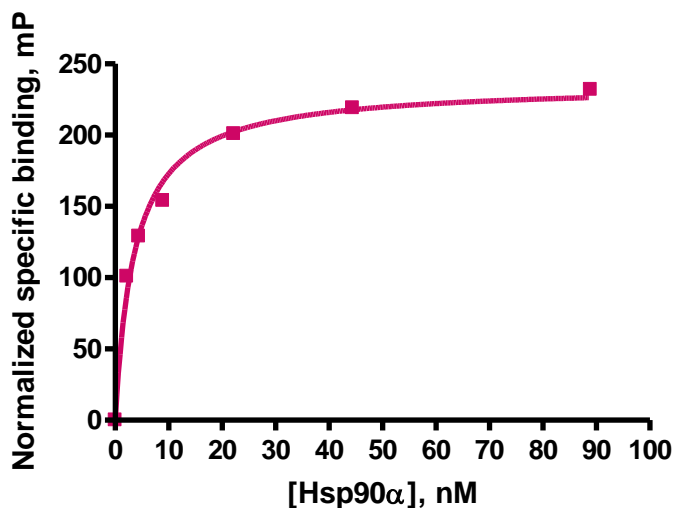
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$$mP = \left(\frac{I_{II} - G(I_1)}{I_{II} + G(I_1)} \right) \times 1000 \quad \text{OR} \quad mP = \left(\frac{G(I_{II}) - I_1}{G(I_{II}) + I_1} \right) \times 1000$$

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

Hsp90 α activity (FITC-geldanamycin binding)



Binding of FITC-geldanamycin to HSP90 α , measured using the Hsp90 α Assay Kit, BPS Bioscience # 50298. Fluorescence was measured at λ_{ex} 485nm, λ_{em} 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*

RELATED PRODUCTS:

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
Hsp90 α recombinant enzyme	50290	200 μ g
Hsp90 β recombinant enzyme	50292	200 μ g
Aha1 recombinant enzyme	50291	200 μ g
Geldanamycin inhibitor	27008	5 mg
MS-275 (Entinostat) inhibitor	27011	25 mg
Hsp90 α Assay Kit (96 well)	50293	96 rxns
Hsp90 β Assay Kit (96 well)	50294	96 rxns
Hsp90 β Assay Kit (384 well)	50299	384 rxns

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