



# SZABO SCANDIC

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**Data Sheet**  
***Hsp90 $\alpha$  N-Terminal Domain Assay Kit***  
Catalog #50293  
Size: 96 reactions

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

The *Hsp90 $\alpha$  N-Terminal Domain Assay Kit* comes in a convenient 96-well format, with enough purified Hsp90 $\alpha$  enzyme, FITC-labeled geldanamycin, and Hsp90 $\alpha$  assay buffer for 100 enzyme reactions. The key to the *Hsp90 $\alpha$  Assay Kit* is the fluorescently labeled geldanamycin. Using this kit, only one simple step on a microtiter plate is required for Hsp90 $\alpha$  reactions. The FITC-labeled geldanamycin is incubated with a sample containing Hsp90 $\alpha$  enzyme to produce a change in fluorescent polarization that can be measured using a fluorescence reader.

**COMPONENTS:**

Catalog #	Component	Amount	Storage	
50290	Hsp90 $\alpha$ recombinant enzyme	100 $\mu$ g	-80°C	<b><i>Avoid freeze/ thaw cycles!</i></b>
50312	FITC-labeled geldanamycin (2.5 $\mu$ M)	30 $\mu$ l	-80°C	
50311	5x Hsp90 assay buffer 1	4 ml	-20°C	
79685	Black, low binding, microtiter plate	1	Room temp.	

**MATERIALS REQUIRED BUT NOT SUPPLIED:**

40 mM DTT (dithiothreitol, also known as Cleland's reagent)  
2 mg/ml BSA (bovine serum albumin)  
Adjustable micropipettor and sterile tips

**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.

**REFERENCE(S):**

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 17 ng/μl (340 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 (15 μl **5x Hsp90 assay buffer 1** + 5 μl 40 mM DTT + 5 μl 2 mg/ml BSA + 40 μl H<sub>2</sub>O). Add 65 μl of master mixture to all wells.

	Blank	Enzyme Positive Control	Enzyme Negative Control	Test Inhibitor
5x Hsp90 assay buffer 1	15 μl	15 μl	15 μl	15 μl
40 mM DTT	5 μl	5 μl	5 μl	5 μl
2 mg/ml BSA	5 μl	5 μl	5 μl	5 μl
H <sub>2</sub> O	40 μl	40 μl	40 μl	40 μl
FITC-Labeled geldanamycin (100 nM)	–	5 μl	5 μl	5 μl
Inhibitor	–	–	–	10 μl
Inhibitor Buffer (no inhibitor)	10 μl	10 μl	10 μl	–
1x HSP90 assay buffer	25 μl	–	20 μl	–
Hsp90α (17 ng/μl)	–	20 μl	–	20 μl
<b>Total</b>	<b>100 μl</b>	<b>100 μl</b>	<b>100 μl</b>	<b>100 μl</b>

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- 4) Add 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 10  $\mu$ l of Inhibitor to each well designated "Test Inhibitor." For the, "Blank", "Enzyme Positive Control" and "Enzyme Negative Control", add 10  $\mu$ l of the same solution without Inhibitor (Inhibitor Buffer).
- 6) Add 20  $\mu$ l of **1x Hsp90 assay buffer** to the well designated "Enzyme Negative Control". Add 25  $\mu$ l **1x Hsp90 assay buffer** to the wells designated "Blank".
- 7) Initiate reaction by adding 20  $\mu$ l of diluted **Hsp90 $\alpha$**  (17 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. Ensure that the machine is set to read the type of plate used in the experiment. 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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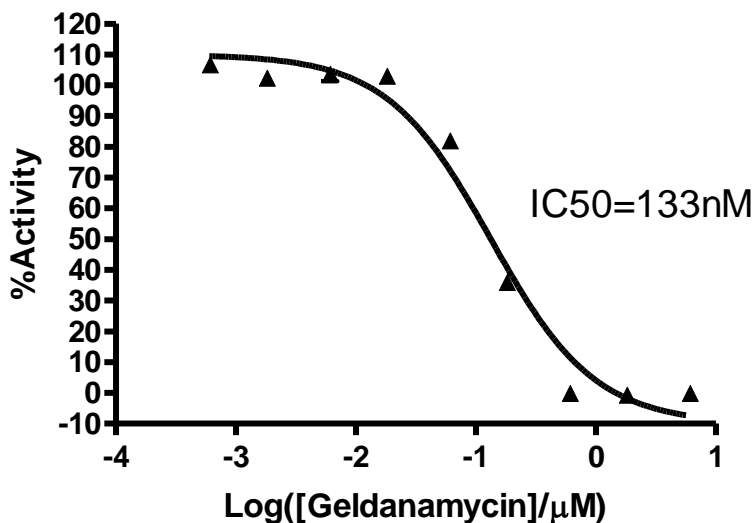
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$$mP = \left( \frac{I_{II} - G(I_L)}{I_{II} + G(I_L)} \right) \times 1000 \quad \text{OR} \quad mP = \left( \frac{G(I_{II}) - I_L}{G(I_{II}) + I_L} \right) \times 1000$$

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

#### Inhibition of HSP90a N-Terminal Domain by Geldanamycin



Inhibition of HSP90 $\alpha$  by geldanamycin, measured using the Hsp90 $\alpha$  Assay Kit, BPS Bioscience #50293. Fluorescence was measured at  $\lambda_{ex}$  485nm,  $\lambda_{em}$  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](mailto:info@bpsbioscience.com)*

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**RELATED PRODUCTS:**

<b><u>Product</u></b>	<b><u>Catalog #</u></b>	<b><u>Size</u></b>
Hsp90 $\alpha$ recombinant enzyme	50290	200 $\mu$ g
Hsp90 $\beta$ recombinant enzyme	50292	200 $\mu$ g
Aha1 recombinant enzyme	50291	200 $\mu$ g
Geldanamycin inhibitor	27008	5 mg
MS-275 (Entinostat) inhibitor	27011	25 mg
Hsp90 $\alpha$ Assay Kit (384 well)	50298	384 rxns
Hsp90 $\beta$ Assay Kit (96 well)	50294	96 rxns
Hsp90 $\beta$ Assay Kit (384 well)	50299	384 rxns

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