



# SZABO SCANDIC

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## Produktinformation



Forschungsprodukte & Biochemikalien



Zellkultur & Verbrauchsmaterial



Diagnostik & molekulare Diagnostik



Laborgeräte & Service

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### Lieferung & Zahlungsart

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### Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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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	<b><i>Avoid freeze/ thaw cycles!</i></b>
50292	Hsp90 $\beta$ recombinant enzyme	140 $\mu$ g	-80°C	
	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°C immediately. *Note: FITC-labeled geldanamycin is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*
- 2) Thaw **Hsp90β** on ice. Upon first thaw, briefly spin tube containing Hsp90β to recover full content of the tube. Aliquot Hsp90β into single use aliquots. Store remaining Hsp90β in aliquots at -80°C immediately. *Note: Hsp90β 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 Hsp90β in 1x Hsp90 assay buffer to 35 ng/μl (350 ng/reaction)\*. Aliquot any remaining enzyme and store undiluted at -70°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 **H<sub>2</sub>O**). 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 <sub>2</sub> 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	–
Hsp90β (35 ng/μl)	–	10 μl	–	10 μl
<b>Total</b>	<b>50 μl</b>	<b>50 μl</b>	<b>50 μl</b>	<b>50 μl</b>

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- 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." 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:

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

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

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

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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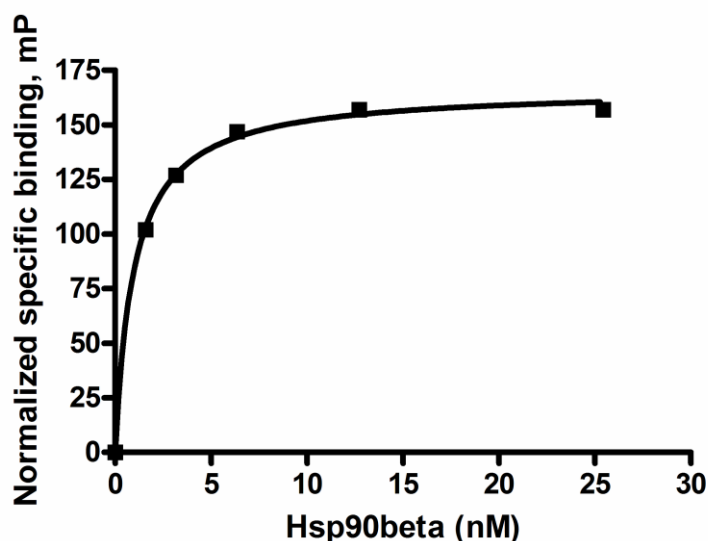


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$$mP = \left( \frac{I_{II} - G(I_L)}{I_{II} + G(I_L)} \right) \times 1000 \quad \text{OR} \quad mP = \left( \frac{G(I_{II}) - I_L}{G(I_{II}) + I_L} \right) \times 1000$$

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:



Binding of FITC-geldanamycin to HSP90 $\beta$ , measured using the Hsp90 $\beta$  Assay Kit, BPS Bioscience # 50299. 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)*

#### RELATED PRODUCTS:

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
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 (96 well)	50293	96 rxns
Hsp90 $\alpha$ Assay Kit (384 well)	50298	384 rxns
Hsp90 $\beta$ Assay Kit (96 well)	50294	96 rxns

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