6044 Cornerstone Court W, Ste E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

Hsp90β N-Terminal Domain Assay Kit

Catalog # 50294
Size: 96 reactions

DESCRIPTION: Hsp90β is a molecular chaperone with essential functions in maintaining transformation. Inhibition of Hsp90β function has been shown to play a role in tumorigenesis and disease progression. The *Hsp90β Assay Kit* is designed for identification of Hsp90β inhibitors using fluorescence polarization. The assay is based on the competition of fluorescently labeled geldanamycin, an HSP90 inhibitor, for binding to purified recombinant Hsp90β.

The *Hsp90β N-Terminal Domain Assay Kit* comes in a convenient 96-well format, with enough purified Hsp90β enzyme, FITC-labeled geldanamycin, and Hsp90β assay buffer for 100 enzyme reactions. The key to the *Hsp90β Assay Kit* is the fluorescently labeled geldanamycin. Using this kit, only one simple step on a microtiter plate is required for Hsp90β reactions. The FITC-labeled geldanamycin is incubated with a sample containing Hsp90β enzyme to produce a change in fluorescent polarization that can then be measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
50292	Hsp90β recombinant enzyme	70 µg	-80°C	***Avoid freeze/ thaw cycles!***
	FITC-labeled geldanamycin (2.5 µM)	30 µl	-80°C	
50311	5x Hsp90 assay buffer 1	4 ml	-20°C	
	Black, low binding NUNC 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 (700 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 (15 µl **5x Hsp90 assay buffer 1** + 5 µl **40 mM DTT** + 5 µl **2 mg/ml BSA** + 40 µl **H_2O**). 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_2O	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	–
Hsp90β (35 ng/µl)	–	20 µl	–	20 µl
Total	**100 µl**	**100 µl**	**100 µl**	**100 µl**

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4) Add 5 µl of diluted **FITC-labeled geldanamycin** (100 nM) to each well designated "Enzyme Positive Control", "Enzyme Negative Control", and "Test Inhibitor."

5) Add 10 µl of **Inhibitor** to each well designated "Test Inhibitor." For the, "Blank", "Enzyme Positive Control" and "Enzyme Negative Control", add 10 µl of the same solution without Inhibitor (**Inhibitor Buffer**).

6) Add 20 µl of **1x HSP90 assay buffer** to the well designated "Enzyme Negative Control". Add 25 µl **1x Hsp90 assay buffer** to the wells designated "Blank".

7) Initiate reaction by adding 20 µl of **diluted Hsp90αβ** (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:

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

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

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

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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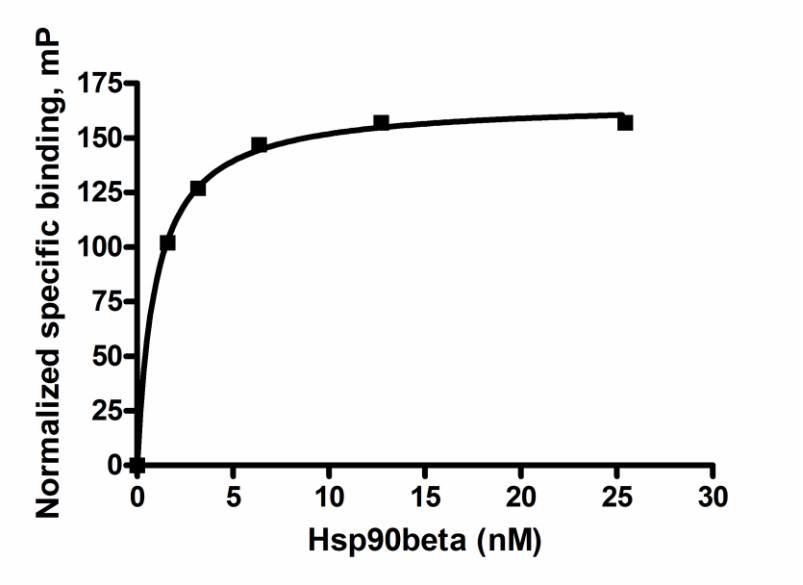
$$mP = \left(\frac{I_{II} - G(I_\perp)}{I_{II} + G(I_\perp)}\right) x\ 1000 \qquad \textbf{OR} \qquad mP = \left(\frac{G(I_{II}) - I_\perp}{G(I_{II}) + I_\perp}\right) x\ 1000$$

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 # 50294. Fluorescence was measured at λex 485nm, λem 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:

Product	Cat. #	Size
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 (384 well)	50299	384 rxns