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Data Sheet

Hsp90α N-Terminal Domain Assay Kit

Catalog #50293
Size: 96 reactions

DESCRIPTION: Hsp90α is a molecular chaperone with essential functions in protein folding and stabilization. Inhibition of Hsp90α function has been shown to play a role in tumorigenesis and disease progression. The *Hsp90α Assay Kit* is designed for identification of Hsp90α 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 Hsp90α.

The Hsp90α N-Terminal Domain Assay Kit comes in a convenient 96-well format, with enough purified Hsp90α enzyme, FITC-labeled geldanamycin, and Hsp90α assay buffer for 100 enzyme reactions. The key to the *Hsp90α Assay Kit* is the fluorescently labeled geldanamycin. Using this kit, only one simple step on a microtiter plate is required for Hsp90α reactions. The FITC-labeled geldanamycin is incubated with a sample containing Hsp90α enzyme to produce a change in fluorescent polarization that can be measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
50290	Hsp90α recombinant enzyme	100 µg	-80°C	***Avoid freeze/ thaw cycles!***
50312	FITC-labeled geldanamycin (2.5 µM)	30 µl	-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_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	–
Hsp90α (17 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 **Hsp90α** (17 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. 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:

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

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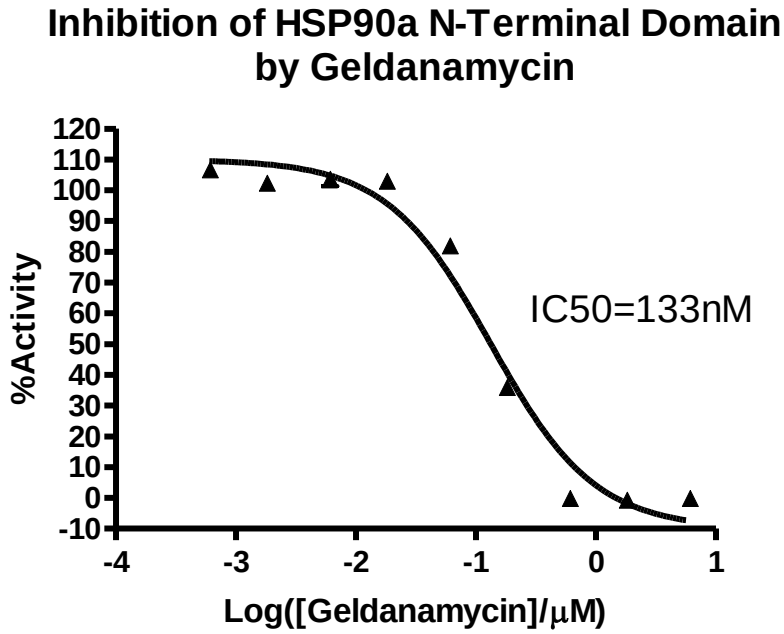
$$mP = \left(\frac{I_{II} - G(I_{\perp})}{I_{II} + G(I_{\perp})}\right) x\ 1000 \qquad \textbf{OR} \qquad mP = \left(\frac{G(I_{II}) - I_{\perp}}{G(I_{II}) + I_{\perp}}\right) x\ 1000$$

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



Inhibition of HSP90α by geldanamycin, measured using the Hsp90α Assay Kit, BPS Bioscience #50293. 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*

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RELATED PRODUCTS:

Product	Catalog #	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 (384 well)	50298	384 rxns
Hsp90β Assay Kit (96 well)	50294	96 rxns
Hsp90β Assay Kit (384 well)	50299	384 rxns

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