

Data Sheet

***Hsp90 α* Assay Kit**

Catalog # w60304
Size: 96 reactions

DESCRIPTION: Hsp90 α is a molecular chaperone with essential functions in protein folding and stabilization. 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 a competitive binding assay, based on the binding of fluorescently labeled geldanamycin, an HSP90 inhibitor, to purified recombinant Hsp90 α .

The *Hsp90 α Assay Kit* comes in a convenient 96-well format, with enough purified Hsp90 α enzyme, FITC-labeled geldanamycin, and Hsp90 α assay buffer for 100 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 that can be measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
w60301	Hsp90 α recombinant enzyme	100 μ g	-80 $^{\circ}$ C	Avoid freeze/ thaw cycles!
	FITC-labeled geldanamycin (2.5 μ M)	30 μ l	-80 $^{\circ}$ C	
	5x Hsp90 assay buffer	4 ml	-20 $^{\circ}$ C	
	Black, low binding NUNC microtiter plate	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

40 mM DTT (dithiothreitol, also known as Cleland's reagent)
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 **Hsp90 α** on ice. Upon first thaw, briefly spin tube containing Hsp90 α to recover full content of the tube. Aliquot Hsp90 α into single use aliquots. Store remaining Hsp90 α in aliquots at -80°C immediately. *Note: Hsp90 α 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 Hsp90 α in 1X Hsp90 assay buffer to 35 ng/ μl (700 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** + 5 μl **40 mM DTT** + 5 μl **2 mg/ml BSA** + 40 μl **H₂O**). Add 65 μl of master mixture to all wells.

	Blank	Enzyme Positive Control	Enzyme Negative Control	Test Inhibitor
5x Hsp90 assay buffer	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 ₂ 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	–
Hsp90 α (35 ng/ μl)	–	20 μl	–	20 μl
Total	100 μl	100 μl	100 μl	100 μl

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- 4) Add 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 10 μ l of **Inhibitor** to each well designated "Test Inhibitor." For the, "Blank", "Enzyme Positive Control" and "Enzyme Negative Control", add 10 μ l of the same solution without Inhibitor (**Inhibitor Buffer**).
- 6) Add 20 μ l of **1x HSP90 assay buffer** to the well designated "Enzyme Negative Control". Add 25 μ l **1x Hsp90 assay buffer** to the wells designated "Blank".
- 7) Initiate reaction by adding 20 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".

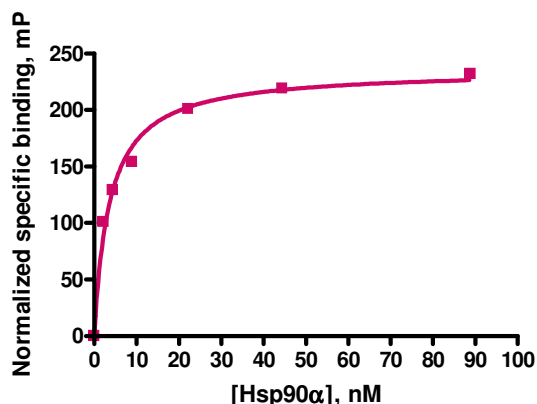
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$$mP = \left(\frac{I_H - G(I_L)}{I_H + G(I_L)} \right) \times 1000 \quad \text{OR} \quad mP = \left(\frac{G(I_H) - I_L}{G(I_H) + I_L} \right) \times 1000$$

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

Hsp90 α activity (FITC-geldanamycin binding)



Binding of FITC-geldanamycin to HSP90 α , measured using the Hsp90 α Assay Kit, Y^hc Bioscience # w60304. 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 West Bioscience, Inc. at sale@westbioscience.com*

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