

## Data Sheet

### ***HSP90α (C-Terminal) Inhibitor Screening Assay Kit***

**Catalog # w60328**  
**Size: 384 reactions**

**DESCRIPTION:** The *HSP90α (C-Terminal) Inhibitor Screening Assay Kit* is designed to measure the inhibition of HSP90α binding to its protein target PPID (also known as Cyclophilin D). The assay kit comes in a convenient AlphaLISA® format, with enough HSP90α (535-732), assay buffer, detection buffer, and purified GST-tagged PPID to perform a total of 384 enzyme reactions. With this kit, only three simple steps on a microtiter plate are required. First, a sample containing HSP90α, PPID, and an inhibitor of choice is incubated for thirty minutes. Next, acceptor beads are added, then donor beads, followed by reading the Alpha-counts.

**COMPONENTS:**

Catalog #	Component	Amount	Storage	
w60327	HSP90α (C-terminal), Biotin-labeled	50 µg	-80 °C	<b>(Avoid freeze/thaw cycles!)</b>
w81106	PPID, GST-tag	100 µg	-80 °C	
	3x HSP90 Assay Buffer 2	4 ml	-20 °C	
	3x HSP90 Detection Buffer	3 ml	-20 °C	

**MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:**

Glutathione AlphaLISA® Acceptor Beads, 5 mg/ml (PerkinElmer #AL109C)  
 AlphaScreen® Streptavidin-conjugated donor beads, 5 mg/ml (PerkinElmer #6760002S)  
 Optiplate-384 (PerkinElmer #6007290)  
 AlphaScreen® microplate reader  
 Adjustable micropipettor and sterile tips

**APPLICATIONS:** Useful for screening inhibitors of HSP90α and for HSP90α binding assays

**CONTRAINDICATIONS:** Only limited amounts of DMSO (< 1%) can be included as it has been shown to disrupt HSP90α:PPID interaction. Green and blue dyes that absorb light in the AlphaScreen® signal emission range (520-620 nm), such as Trypan Blue. Avoid the use of the potent singlet oxygen quenchers such as sodium azide (NaN<sub>3</sub>) or metal ions (Fe<sup>2+</sup>, Fe<sup>3+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup> and Ni<sup>2+</sup>). The presence of >1% RPMI 1640 culture medium leads to a signal reduction due to the presence of excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen® assays.

**STABILITY:** At least one year from date of receipt when stored as directed.

**REFERENCE(S):** Allan, R.K. *et al. J. Biol.Chem* 2006 **281(11)**: 7161-71.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

## ASSAY PROTOCOL:

All samples and controls should be tested in duplicate. Use slow shaking for all incubations.

### Step 1:

- 1) Thaw **HSP90 $\alpha$**  and **PPID** on ice. Upon first thaw, briefly spin tube containing protein to recover full content of the tube. Aliquot protein into single use aliquots. Store remaining undiluted protein in aliquots at -80°C immediately. *Note: the HSP90 $\alpha$  and PPID proteins are very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*
- 2) Dilute **3x HSP90 Assay Buffer 2** with water to **1x HSP90 Assay Buffer 2**.
- 3) Dilute **HSP90 $\alpha$**  in **1x HSP90 Assay Buffer 2** at 1.5 ng/ $\mu$ l. Add 4  $\mu$ l of diluted HSP $\alpha$  protein to each well designated for the "Positive Control", "Test Inhibitor", and "Blank". To the wells labeled "Substrate Control", add 4  $\mu$ l of **1x HSP90 Assay Buffer 2**. Discard any unused diluted protein after use.
- 4) Add 2  $\mu$ l of inhibitor solution to each well designated "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 2  $\mu$ l of the same solution without inhibitor (inhibitor buffer). *Note: Keep DMSO concentration below 1%.*
- 5) Dilute **PPID** in **1x HSP90 Assay Buffer 2** at 10 ng/ $\mu$ l. Keep diluted protein on ice until use.
- 6) Add 4  $\mu$ l of **1x HSP90 Assay Buffer 2** to the well designated "Blank".
- 7) Initiate reaction by adding 4  $\mu$ l of diluted **PPID** to each well designated for the "Substrate control", "Positive Control", and "Test Inhibitor". Incubate at room temperature for 30 minutes.

	Blank	Substrate Control	Positive Control	Test Inhibitor
Diluted HSP90 $\alpha$ (1.5 ng/ $\mu$ l)	4 $\mu$ l	–	4 $\mu$ l	4 $\mu$ l
Test Inhibitor/Activator	–	–	–	2 $\mu$ l
Inhibitor buffer (no inhibitor)	2 $\mu$ l	2 $\mu$ l	2 $\mu$ l	–
1x HSP90 Assay Buffer 2	4 $\mu$ l	4 $\mu$ l	–	–
Diluted PPID (10 ng/ $\mu$ l)	–	4 $\mu$ l	4 $\mu$ l	4 $\mu$ l
<b>Total</b>	<b>10 <math>\mu</math>l</b>	<b>10 <math>\mu</math>l</b>	<b>10 <math>\mu</math>l</b>	<b>10 <math>\mu</math>l</b>

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

## Step 2:

**Note: Protect your samples from direct exposure to light!**

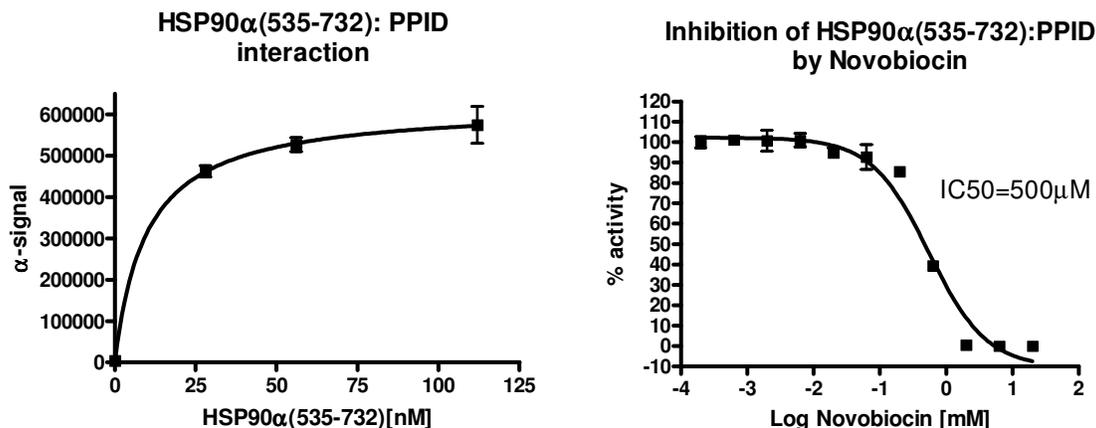
- 1) Dilute **3x Detection Buffer** with water to **1x Detection Buffer**. Dilute **Glutathione AlphaLISA<sup>®</sup> Acceptor Beads** (PerkinElmer #AL109C) 250-fold with **1x Detection Buffer**. Add 10  $\mu$ l per well. Shake plate briefly. Incubate at room temperature for 30 minutes.

## Step 3:

- 1) Dilute **Streptavidin-conjugated donor beads** (PE #6760002S) 125-fold with **1x Detection Buffer**. Add 10  $\mu$ l per well. Incubate at room temperature for 1 hour.
- 2) Read Alpha-counts.

Due to lot to lot variability in AlphaScreen<sup>®</sup> bead performance, it may be necessary to optimize assay conditions. For example, slight adjustments to HSP90 $\alpha$  or PPID concentrations may improve signal-to-noise ratio.

## EXAMPLE OF ASSAY RESULTS:



HSP90 $\alpha$  (C-terminal):PPID binding activity measured using *HSP90 $\alpha$  (C-Terminal) Inhibitor Screening Assay Kit*, West Bioscience, Catalog # w60328. Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at [sale@westbioscience.com](mailto:sale@westbioscience.com).

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.



9891 Irvine Center Dr. Suite 200

Irvine, CA 92618

United States

**Tel:** 1.800.831.1518

**Fax:** 1.800.831.1518

**Email:** [sale@westbioscience.com](mailto:sale@westbioscience.com)

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

[www.westbioscience.com](http://www.westbioscience.com)