

Data Sheet

HSP90α (C-Terminal) Inhibitor Screening Assay Kit

Catalog # 50317

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	
50316	HSP90α (C-terminal), Biotin-labeled*	5 µg	-80°C	(Avoid freeze/ thaw cycles!)
71095	PPID, GST-tag*	2 x 10 µg	-80°C	
50324	3x HSP90 Assay Buffer 2	4 ml	-20°C	
	3x HSP90 Detection Buffer	3 ml	-20°C	

*The concentration of HSP90 and PPID are lot-specific and will be indicated on the tubes containing the protein

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 (NaN₃) or metal ions (Fe²⁺, Fe³⁺, Cu²⁺, Zn²⁺ and Ni²⁺). 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.

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ASSAY PROTOCOL:

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

Step 1:

- 1) Thaw **HSP90 α** 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 α 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 α** in **1x HSP90 Assay Buffer 2** at 1.5 ng/ μ l. Add 4 μ l of diluted HSP α protein to each well designated for the “Positive Control”, “Test Inhibitor”, and “Blank”. To the wells labeled “Substrate Control”, add 4 μ l of **1x HSP90 Assay Buffer 2**. Discard any unused diluted protein after use.
- 4) Add 2 μ l of inhibitor solution to each well designated “Test Inhibitor”. For the “Positive Control”, “Substrate Control” and “Blank”, add 2 μ 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/ μ l. Keep diluted protein on ice until use.
- 6) Add 4 μ l of **1x HSP90 Assay Buffer 2** to the well designated “Blank”.
- 7) Initiate reaction by adding 4 μ 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 α (1.5 ng/ μ l)	4 μ l	–	4 μ l	4 μ l
Test Inhibitor/Activator	–	–	–	2 μ l
Inhibitor buffer (no inhibitor)	2 μ l	2 μ l	2 μ l	–
1x HSP90 Assay Buffer 2	4 μ l	4 μ l	–	–
Diluted PPID (10 ng/ μ l)	–	4 μ l	4 μ l	4 μ l
Total	10 μl	10 μl	10 μl	10 μl

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Step 2:

Note: Protect your samples from direct exposure to light!

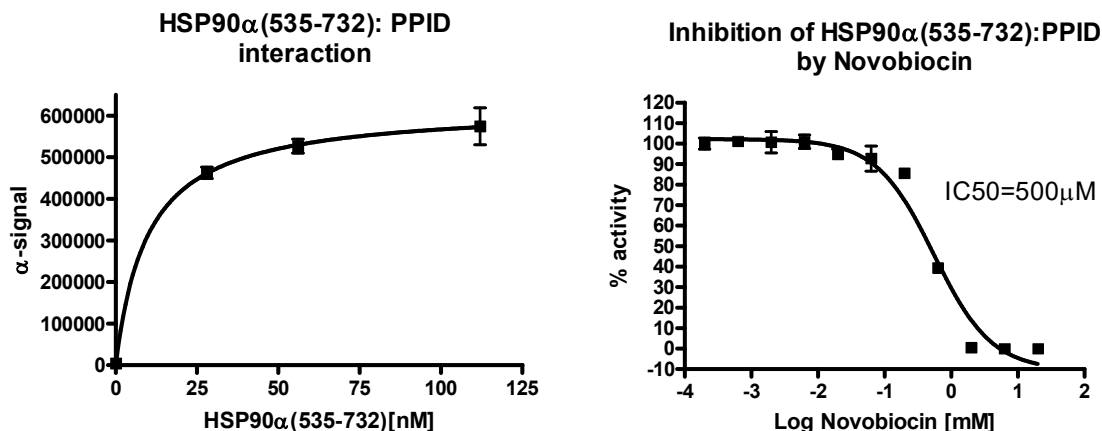
- 1) Dilute **3x Detection Buffer** with water to **1x Detection Buffer**. Dilute **Glutathione AlphaLISA® Acceptor Beads** (PerkinElmer #AL109C) 250-fold with **1x Detection Buffer**. Add 10 μ l per well. Shake plate briefly. Incubate at room temperature for 30 minutes.

Step 3:

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

Due to lot to lot variability in AlphaScreen® bead performance, it may be necessary to optimize assay conditions. For example, slight adjustments to HSP90 α or PPID concentrations may improve signal-to-noise ratio.

EXAMPLE OF ASSAY RESULTS:



HSP90 α (C-terminal):PPID binding activity measured using *HSP90 α (C-Terminal) Inhibitor Screening Assay Kit*, BPS Bioscience, Catalog # 50317. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

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RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
HSP90 α (C-terminal), Biotin-labeled	50316	100 μ g
HSP90 β (C-terminal), Biotin-labeled	50313	100 μ g
HSP90 α recombinant enzyme	50290	200 μ g
HSP90 β recombinant enzyme	50292	200 μ g
Aha1 recombinant enzyme	50291	200 μ g
Geldanamycin inhibitor	27008	5 mg
MS-275 (Entinostat) inhibitor	27011	25 mg
Novobiocin inhibitor	27501	250 μ l
HSP90 α Assay Kit (96 well)	50293	96 rxns
HSP90 β Assay Kit (96 well)	50294	96 rxns
HSP90 α Assay Kit (384 well)	50298	384 rxns
HSP90 β Assay Kit (384 well)	50299	384 rxns

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