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Data Sheet

HSP90β (C-Terminal) Inhibitor Screening Assay Kit

Catalog # 50314
Size: 384 reactions

DESCRIPTION: The *HSP90β (C-Terminal) Inhibitor Screening Assay Kit* is designed to measure the inhibition of HSP90β binding to its protein target PPID (also known as Cyclophilin D). The assay kit comes in a convenient AlphaLISA® format, with enough HSP90β (527-724), 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 HSP90β, 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	
50313	HSP90β (C-terminal), Biotin-labeled	100 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
71095	PPID, GST-tag	100 µg	-80°C	
50324	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 HSP90β and for HSP90β binding assays

CONTRAINDICATIONS: Only limited amounts of DMSO (< 0.5%) can be included as it has been shown to disrupt HSP90β: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_3) or metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} and Ni^{2+}). 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 **HSP90β** 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 HSP90β and PPID proteins are very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

2) Dilute **HSP90β** in **1x HSP90 Assay Buffer 2** at 140 ng/µl. Dilute **PPID** in **1x HSP90 Assay Buffer 2** at 75 ng/µl. Keep diluted proteins on ice until use. Discard any unused diluted protein after use.

3) Prepare the master mixture: N wells × (2.5 µl **3× HSP90 Assay Buffer 2** + 1 µl diluted **HSP90β** + 0.5 µl H_2O).

4) Add 4 µl of master mixture to each well designated for the "Positive Control", "Test Inhibitor", and "Blank". To the wells labeled "Substrate Control", add (2.5 µl **3× HSP90 Assay Buffer 2** + 1.5 µl H_2O).

	Blank	Substrate Control	Positive Control	Test Inhibitor
3x HSP90 Assay Buffer 2	2.5 µl	2.5 µl	2.5 µl	2.5 µl
H_2O	0.5 µl	1.5 µl	0.5 µl	0.5 µl
Diluted HSP90β (140 ng/µl)	1 µl	–	1 µl	1 µl
Test Inhibitor/Activator	–	–	–	2.5 µl
Inhibitor buffer (no inhibitor)	2.5 µl	2.5 µl	2.5 µl	–
1x HSP90 Assay Buffer 2	3.5 µl			
PPID (75 ng/µl)	–	3.5 µl	3.5 µl	3.5 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 l**

5) Add 2.5 µl of inhibitor solution to each well designated "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 2.5 µl of the same solution without inhibitor (inhibitor buffer). *Note: Keep DMSO concentration below 0.5%.*

6) Add 3.5 µl of **1x HSP90 Assay Buffer 2** to the well designated "Blank".

7) Initiate reaction by adding 2.5 µl of diluted **PPID** prepared as described above. Incubate at room temperature for 30 minutes.

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Step 2:

Note: Protect your samples from direct exposure to light!

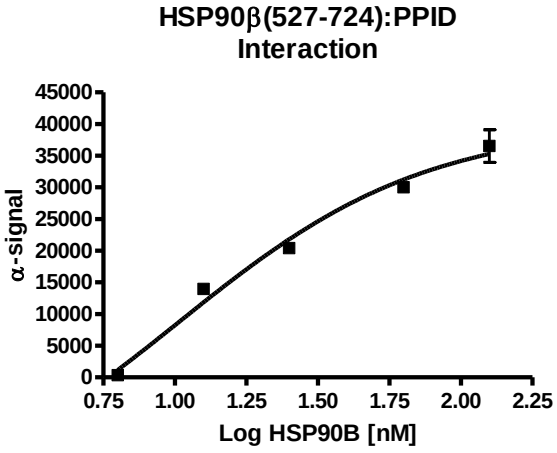
1) Dilute **Glutathione AlphaLISA® Acceptor Beads** (PerkinElmer #AL109C) 250-fold with **1x Detection Buffer**. Add 10 µl per well. Shake plate briefly. Incubate at room temperature for 30 minutes.

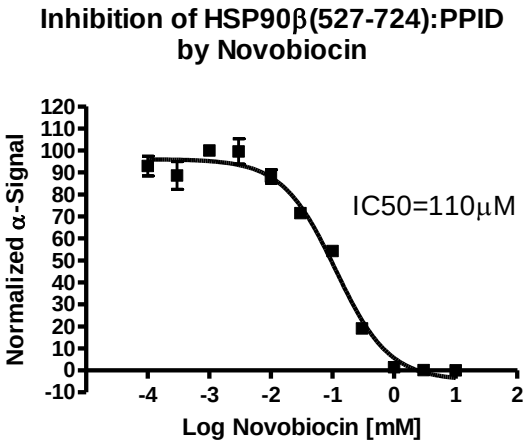
Step 3:

1) Dilute **Streptavidin-conjugated donor beads** (PE #6760002S) 125-fold with **1x Detection Buffer.** Add 10 µl 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 bromodomain or ligand concentrations may improve signal-to-noise ratio.*

EXAMPLE OF ASSAY RESULTS:





HSP90β (C-terminal):PPID binding activity measured using *HSP90β (C-Terminal) Inhibitor Screening Assay Kit*, BPS Bioscience, Catalog # 50314. *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 Name	Catalog #	Size
HSP90β (C-terminal), Biotin-labeled	50313	100 µg
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
Novobiocin inhibitor	27501	250 µl
HSP90α Assay Kit (96 well)	50293	96 rxns
HSP90β Assay Kit (96 well)	50294	96 rxns
HSP90α Assay Kit (384 well)	50298	384 rxns
HSP90β Assay Kit (384 well)	50299	384 rxns