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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet
HSP90α C-Terminal Domain TR-FRET Assay Kit
Catalog # 50289

DESCRIPTION:
This assay kit is designed to measure the inhibition of the HSP90α binding to its protein target PPID (also known as Cyclophilin D) in a homogeneous, 96 reaction format. This assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications. The assay procedure is straightforward and simple. A sample containing terbium-labeled donor, dye-labeled acceptor, HSP90α, substrate, and an inhibitor is incubated for 2 hours. The protein-protein interaction is then assayed by measuring the TR-FRET between the PPID and HSP90α using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
50316	HSP90α (C-Terminal), Biotin Labeled	5 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
71095	PPID, GST-tag	5 µg	-80°C	
	Tb donor	10 µl	-20°C	
	Dye-labeled acceptor	10 µl	-20°C	
50324	3x HSP90 Assay Buffer 2	4 ml	-20°C	
79685	Black-96 Well Plate	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)Adjustable micropipettor and sterile tips

APPLICATIONS: Great for screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: At least 6 months from date of receipt when stored as directed.

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

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All samples and controls should be tested in duplicate.

1) Dilute one part **3x HSP90 Assay Buffer 2** with 2 parts distilled water (3-fold dilution) to make **1x HSP90 Assay Buffer 2**. Make only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C.

2) Dilute **Tb-labeled donor** and **Dye-labeled acceptor** 100-fold in **1x HSP90 Assay Buffer 2**. Make only sufficient quantities needed for the assay; store remaining stock solution in aliquots at -20°C.

3) Add 10 µl of diluted **Tb-labeled donor**, and 10 µl of diluted **Dye-labeled acceptor** to every well.

4) Add 4 µl of inhibitor solution to each well designated "Test Inhibitor." Add 4 µl of the same solution without inhibitor (inhibitor buffer) to the wells labeled "Substrate Control," and "Positive Control."

	Positive Control	**Negative Control**	**Test Inhibitor**
Tb-labeled donor	10 µl	10 µl	10 µl
Dye-labeled acceptor	10 µl	10 µl	10 µl
Test Inhibitor	–	–	4 µl
Inhibitor Buffer (no inhibitor)	4 µl	4 µl	–
Diluted PPID (3 ng/µl)	10 µl	–	10 µl
1x HSP90 Assay Buffer	–	10 µl	–
HSP90α (2 ng/µl)	6 µl	6 µl	6 µl
Total	**40 µl**	**40 µl**	**40 µl**

5) Thaw **HSP90**α (C-Terminal), Biotin Labeled **and PPID, GST-tag** on ice. Upon first thaw, briefly spin tube containing ligand to recover the full contents of the tube. Aliquot each ligand into single-use aliquots. Store remaining undiluted ligand at −80°C immediately. *Note: the proteins are very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots.*

6) Dilute **PPID, GST-tag** in **1x HSP90 Assay Buffer 2** to 3 ng/µL (30 ng/reaction). Add 10 µl of diluted PPID to each well designated as "Positive Control" and "Test Inhibitor". Add 10 µl of **1x HSP90 Assay Buffer 2** to the wells labeled "Negative Control".

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7) Dilute HSP90α in **1x HSP90 Assay Buffer 2** to 2 ng/µl (12 ng/reaction). Initiate reaction by adding 6 µl of diluted **HSP90α** to every well. Discard any remaining diluted HSP90α protein after use.

8) Incubate at room temperature for 2 hours *(keep out of direct light)*.

9) Read the fluorescent intensity in a microtiter-plate reader capable of TR-FRET.

Instrument Settings

Reading Mode	Time Resolved
Excitation Wavelength	340±20 nm
Emission Wavelength	620±10 nm
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	340±20 nm
Emission Wavelength	665±10 nm
Lag Time	60 µs
Integration Time	500 µs

(Sample data was collected on a Tecan Infinite M1000 Pro. Gain was set as optimal and Z' was determined from a 100 percent binding well.)

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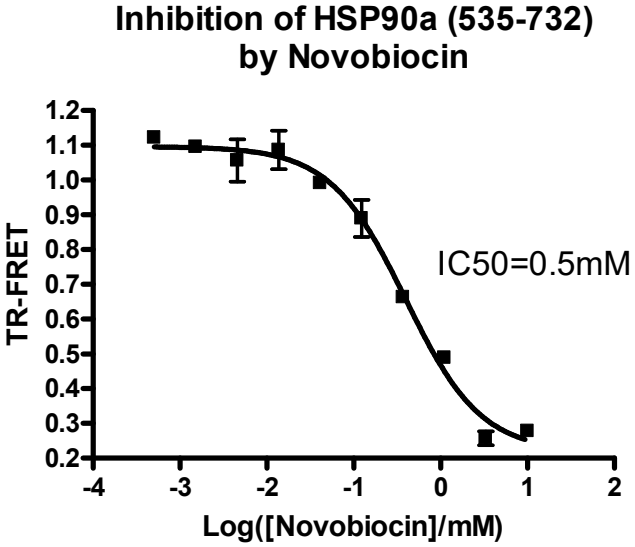
CALCULATING RESULTS:
Two sequential measurements should be conducted. Tb-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

When percentage activity is calculated, the FRET value from the negative control can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

$$\% \text{ Activity} = \frac{FRET_s - FRET_{neg}}{FRET_P - FRET_{neg}} \times 100\%$$

Where $FRET_s$ = Sample FRET, $FRET_{neg}$ = negative control FRET, and $FRET_P$ = Positive control FRET.

EXAMPLE OF ASSAY RESULTS:



Interaction of HSP90a C-Terminal Domain (BPS Bioscience Cat. #50316) with BET Ligand. Assay was done according to protocol for the HSP90a C-Terminal Domain, TR-FRET Assay Kit (BPS Cat. #50289). *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	50290	200 µg
HSP90β	50292	200 µg
HSP90α (C-terminal Domain), Biotin-labeled	50316	100 µg
PPID (CYP-40)	71095	100 µg
Novobiocin	27501	250 µl
HSP90α (C-terminal Domain) Screening Kit	50317	384 rxns
HSP90β (C-terminal Domain) Screening Kit	50314	384 rxns
HSP90α Assay Kit	50293	96 rxns
HSP90α Assay Kit	50298	384 rxns
HSP90β Assay Kit	50294	96 rxns
HSP90β Assay Kit	50299	96 rxns
HSP90β (C-terminal Domain) TR-FRET Kit	50262	384 rxns

Note: Tb-labeled donor and dye-labeled acceptor are products of Cisbio Bioassays.

To place your order, please contact us by Phone **1.858.202.1401** Fax **1.858.481.8694**

Or you can Email us at: **info@bpsbioscience.com**