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- Trockeneiszuschlag
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Data Sheet
HSP90 β (C-Terminal Domain) TR-FRET Assay Kit
Catalog # 50262

DESCRIPTION:

The HSP90 β (C-Terminal) TR-FRET assay kit is designed to measure the inhibition of HSP90 α binding to its protein target PPID (also known as Cyclophilin D or Cyp40) in a homogeneous, 384 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, HSP90 β , 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 HSP90 β using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
50313	HSP90 β (C-Terminal Domain), Biotin Labeled	10 μ g	-80 $^{\circ}$ C	(Avoid freeze/ thaw cycles!)
71095	PPID, GST-tag	10 μ g	-80 $^{\circ}$ C	
	Tb donor	20 μ l	-20 $^{\circ}$ C	
	Dye-labeled acceptor	20 μ l	-20 $^{\circ}$ C	
50324	3x HSP90 Assay Buffer 2	4 ml	-20 $^{\circ}$ C	
	White, Nonbinding, low volume, microtiter 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

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

- 1) Dilute one part **3x HSP90 TR-FRET Assay Buffer** with 2 parts distilled water (3-fold dilution) to make **1x HSP90 Assay Buffer**. 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 Buffer**. Make only sufficient quantities needed for the assay; store remaining stock solution in aliquots at -20°C.
- 3) Add 5 µl of diluted **Tb-labeled donor**, and 5 µl of diluted **Dye-labeled acceptor** to every well.
- 4) Add 2 µl of inhibitor solution to each well designated "Test Inhibitor". Add 2 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	5 µl	5 µl	5 µl
Dye-labeled acceptor	5 µl	5 µl	5 µl
Test Inhibitor	-	-	2 µl
Inhibitor Buffer (no inhibitor)	2 µl	2 µl	-
PPID (3 ng/µl)	5 µl	-	5 µl
1x HSP90 Buffer	-	5 µl	-
HSP90β (2 ng/µl)	3 µl	3 µl	3 µl
Total	20 µl	20 µl	20 µl

- 5) Thaw **HSP90β** and **PPID** 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** in **1x HSP90 Assay Buffer** to 3 ng/µl (15 ng/reaction). Add 5 µl of diluted **PPID** to each well designated as "Positive Control" and "Test Inhibitor". Add 5 µl of **1x HSP90 Buffer 2** to the wells labeled "Negative Control".
- 7) Dilute **HSP90β** in **HSP90 Buffer 2** to 2 ng/µl (6 ng/reaction). Initiate reaction by adding 3 µl of diluted **HSP90β** to every well. Discard any remaining diluted **HSP90β** protein after use.
- 8) Incubate at room temperature for 2 hours.

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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

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.

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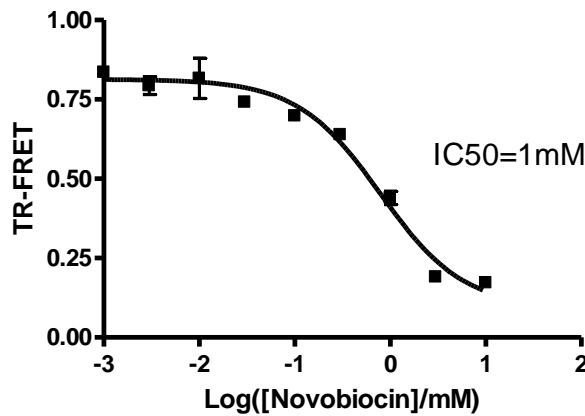
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EXAMPLE OF ASSAY RESULTS:

**Inhibition of HSP90b (527-724):PPID
 by Novobiocin**



Inhibition of HSP90 β C-Terminal Domain (BPS Bioscience Cat. #50313) interaction with PPID by Novobiocin. Assay was done according to protocol for the HSP90 β C-Terminal Domain, TR-FRET Assay Kit (BPS Cat. #50262). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog</u>	<u>Size</u>
HSP90 α	50290	200 μ g
HSP90 β	50292	200 μ g
HSP90 β (C-terminal Domain), Biotin-labeled	50313	100 μ g
PPID (CYP-40)	71095	100 μ g
Novobiocin	27501	250 μ l
HSP90 α (C-terminal Domain) Inhibitor Screening Assay Kit	50317	384 rxns
HSP90 β (C-terminal Domain) Inhibitor Screening Assay Kit	50314	384 rxns
HSP90 α Assay Kit	50293	96 rxns
HSP90 α Assay Kit	50298	384 rxns
HSP90 β Assay Kit	50294	96 rxns
HSP90 β Assay Kit	50299	384 rxns
HSP90 α (C-terminal Domain) TR-FRET Assay Kit	50261	384 rxns

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

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