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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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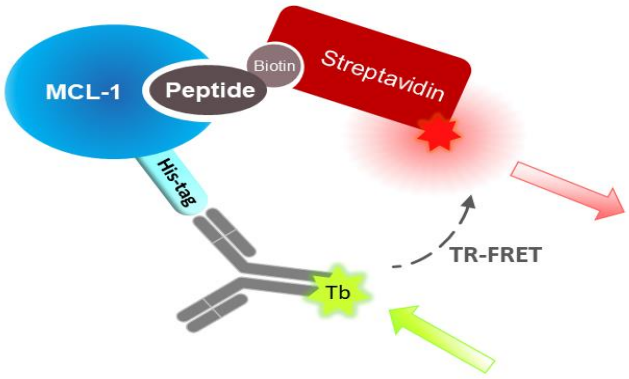

Figure 1: MCL-1 TR-FRET Assay Kit.

Description

The Induced myeloid leukemia cell differentiation protein (MCL-1) TR-FRET Assay Kit (Rat) is designed to measure the inhibition of rat MCL-1 binding to its ligand in a homogeneous 384-well format. This TR-FRET based assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications. The assay is straightforward and fast. The TR-FRET signal is measured after a 1-hour incubation of the anti-His terbium (Tb)-labeled donor, dye-labeled acceptor, recombinant Rat MCL-1 protein, peptide ligand, and the inhibitor of interest.

Background

Induced myeloid leukemia cell differentiation protein (MCL-1) is part of the BCL-2 family of proteins that regulates apoptosis. The intrinsic (mitochondrial) apoptotic pathway is activated by intracellular signals strictly controlled by the BCL-2 family of proteins and are grouped into the antiapoptotic, activator, and sensitizer subsets. All function through complex interactions to regulate the integrity of the mitochondrial membrane. As part of the antiapoptotic subset, MCL-1 inhibits mitochondrial outer membrane permeabilization (MOMP) and the release of cytochrome C from mitochondria. MCL-1 is essential for the survival of various cell types that include the nervous system, cardiomyocytes and lymphocytes. Overexpression of MCL-1 is frequently observed in many tumor types and is closely associated with tumorigenesis and drug resistance. Because of its ability to strictly regulate cell fate, MCL-1 is a target of interest for many drug therapies.

Application(s)

Screen for inhibitors of the interaction between Rat MCL-1 and MCL-1 peptide ligand.

Supplied Materials

Catalog #	Name	Amount	Storage
101299	Rat MCL-1*	5 µg	-80°C
79507	MCL-1 Peptide Ligand	400 reactions	-80°C
30017	Anti-His Tb-labeled donor	2 x 10 µl	-20°C
	Dye-labeled acceptor	2 x 10 µl	-20°C
	3x MCL TR-FRET Assay Buffer	4 ml	-20°C
79969	384-well white microplate	1	Room Temp.

**The initial concentration of Rat MCL-1 is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- Fluorescence microplate reader capable of measuring Time Resolved-Fluorescence Resonance Energy Transfer (TR-FRET)
- Adjustable micropipettor and sterile tips

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed. **Avoid multiple freeze/thaw cycles**.

Safety

This product is for research purposes only and not for human or therapeutic use. **The TR-FRET detection reagent contains a toxic compound**. **Use appropriate precautions.** This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

Keep final DMSO concentration at or below 1%.

Assay Protocol

All samples and controls should be tested in duplicate. We recommend preincubating antibodies or protein inhibitors with the target protein. For small molecule inhibitors, pre-incubation may also be beneficial, depending on the experimental conditions.

Step 1:

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

2. Dilute Anti-His Tb-labeled donor and Dye-labeled acceptor 100-fold in 1x MCL TR-FRET Assay Buffer. Make only sufficient quantities as needed for the assay; store the remaining stock solution in aliquots at -20°C.

3. Add 5 µl of diluted Anti-His Tb-labeled donor, and 5 µl of diluted Dye-labeled acceptor to each well designated "Test Inhibitor," "Negative Control," and "Positive Control."

4. Prepare the Test Inhibitor (2 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 20 µl.

    a) If the Test Inhibitor is water-soluble, prepare serial dilutions in the Assay Buffer, 10-fold more concentrated than the desired final concentrations. For the positive and negative controls, use 1x Assay Buffer (Diluent Solution).

    OR

    b) If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in Assay Buffer to prepare the highest concentration of the intermediate dilutions. The concentration of DMSO is now 10%.

    c) Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in Assay Buffer to keep the concentration of DMSO constant.

    d) For positive and negative controls, prepare 10% DMSO in Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

5. Add 2 µl of test inhibitor solution to each well designated "Test Inhibitor." Add 2 µl of Diluent Solution to the wells labeled "Negative Control" and "Positive Control."

6. Resuspend MCL-1 Peptide Ligand in 320 µl of 1x MCL TR-FRET Assay Buffer. Briefly spin the tube containing the peptide to recover the full contents of the tube.

    a) If the assay plate is going to be used more than once, prepare enough for this portion of the assay and aliquot the remaining peptide into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C.

    Note: The Peptide Ligand is sensitive to freeze/thaw cycles. Do not re-use thawed aliquots.

7. Dilute the solution of MCL-1 Peptide Ligand 10-fold using 1x MCL TR-FRET Assay Buffer (i.e., Add 9 µl of 1x MCL TR-FRET Assay Buffer to 1 µl of resuspended MCL-1 Peptide Ligand).

8. Add 5 µl of this diluted MCL-1 Peptide Ligand to each well designated as "Positive Control" and "Test Inhibitor."

9. Add 5 µl of 1x MCL TR-FRET Assay Buffer to the wells labeled as "Negative Control."

10. Thaw Rat MCL-1 protein on ice. Briefly spin the tube containing the protein to recover the full contents of the tube. If the assay plate is going to be used more than once, prepare enough enzyme for this portion of the assay and aliquot the remaining protein into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C.

    Note: Rat MCL-1 is very sensitive to freeze/thaw cycles. Do not reuse thawed aliquots or diluted protein.

11. Dilute Rat MCL-1 in 1x MCL TR-FRET Assay Buffer to 4.17 ng/µl (12.5 ng/reaction). Initiate the reaction by adding 3 µl of diluted MCL-1 to the wells designated as "Negative Control," "Positive Control," and "Test Inhibitor." Discard any remaining diluted Rat MCL-1 protein after use.

Component	**Negative Control**	**Positive Control**	**Test Inhibitor**
Anti-His Tb-labeled donor	5 µl	5 µl	5 µl
Dye-labeled acceptor	5 µl	5 µl	5 µl
Test Inhibitor	-	-	2 µl
Diluent solution (no inhibitor)	2 µl	2 µl	-
1x MCL-1 TR-FRET Buffer	5 µl	-	-
Diluted MCL-1 Peptide Ligand	-	5 µl	5 µl
Rat MCL-1 (4.17 ng/µl)	3 µl	3 µl	3 µl
Total	**20 µl**	**20 µl**	**20 µl**

12. Incubate the plate at room temperature for 2 hours.

13. Read the TR-FRET signal in a microtiter-plate reader under settings described below (settings may need optimization depending on the instrument). The "Blank" value is subtracted from all other values.

Channel	Variable	Recommended Value
1	Excitation wavelength (nm)	340 ± 20
	Emission wavelength (nm)	620 ± 10
	Lag time (µs)	60
	Integration time (µs)	500
2	Excitation wavelength (nm)	340 ± 20
	Emission wavelength (nm)	665 ± 10
	Lag time (µs)	60
	Integration time (µs)	500

Example Results

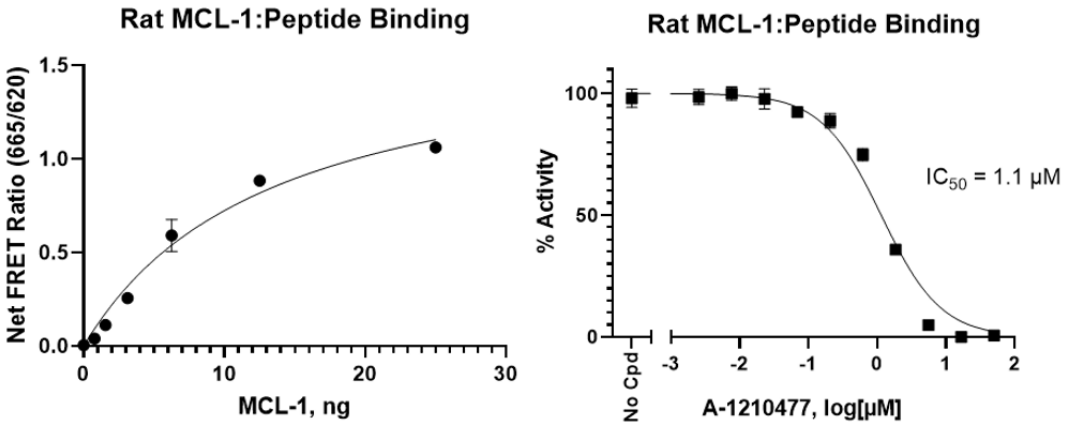

Figure 2: Binding of Rat MCL-1.
Titration of Rat MCL-1 (left) and inhibition of Rat MCL-1:Peptide binding by A-1210477 (right) using the MCL-1 TR-FRET Assay Kit (Rat) (BPS Bioscience #78804). Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

General Considerations

"Positive Control":

The "Positive Control" is the maximum signal determined upon the addition of diluent solution (for example, 1% DMSO in 1x MCL TR-FRET Buffer) in the absence of inhibitor.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Reference

Wang, H., *et al*. Targeting MCL-1 in cancer: current status and perspectives. J Hematol Oncol 14, 67 (2021) Abid, M., *et al. Curr Med Chem*, 2017; **24:**4488-4514.

Related Products

Products	*Catalog #*	*Size*
MCL-1 TR-FRET Assay Kit	79506	384 rxns
MCL-1 TR-FRET Assay Kit (Mouse)	79928	384 rxns
MCL1, His-Tag (Guinea Pig) Recombinant	101311	100 µg