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Data Sheet
MCL-1 TR-FRET Assay Kit (Mouse)
Catalog #79928
Size: 384 reactions

DESCRIPTION: The Mouse MCL-1 TR-FRET Assay Kit is designed to measure the inhibition of mouse MCL-1 binding to its ligand in a homogeneous 384 reaction format. This TR-FRET-based assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications. The assay procedure is straightforward and simple; TR-FRET signal can be measured after 1-hour incubation of a sample containing terbium (Tb)-labeled donor, dye-labeled acceptor, recombinant Mouse MCL-1 protein, peptide ligand, and an inhibitor.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100637	Mouse MCL-1	10 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
79507	MCL-1 Peptide Ligand	400 rxns	-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	
	White, nonbinding, low volume, microtiter plate	1	Room temp.	

MATERIALS 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):

1. Abid, M., *et al*. *Curr Med Chem*, 2017; **24:**4488-4514.

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

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 needed for the assay; store 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 needed for the assay; store 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) 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 "Negative Control" and "Positive Control."

	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
Inhibitor Buffer (no inhibitor)	2 µl	2 µl	–
1x MCL-1 TR-FRET Assay Buffer	5 µl	–	–
MCL-1 Peptide Ligand	–	5 µl	5 µl
Mouse MCL-1 (8.33ng/µl)	3 µl	3 µl	3 µl
Total	**20 µl**	**20 µl**	**20 µl**

Note: The Mouse MCL1 TR-FRET Assay Kit is compatible with up to 0.5% final DMSO concentration.

5) Resuspend a tube with **MCL-1 Peptide Ligand** in **800** µl of **1x MCL TR-FRET Assay Buffer**. Aliquot **MCL-1 Peptide Ligand** into single-use aliquots. Store remaining diluted ligand at −80°C immediately. *Note: Ligand is sensitive to freeze/thaw cycles. Do not re-use thawed aliquots.*

6) Add 5 µL of diluted **MCL-1 Peptide Ligand** to each well designated as "Positive Control" and "Test Inhibitor." Add 5 µl of **1x MCL TR-FRET Assay Buffer** to the wells labeled as "Negative Control."

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7) Thaw **Mouse MCL-1** protein on ice. Upon first thaw, briefly spin tube containing protein to recover the full contents of the tube. Aliquot **Mouse MCL-1** protein into single-use aliquots. Store remaining undiluted aliquots at −80°C immediately. *Note: Mouse MCL-1 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

8) Dilute **Mouse MCL-1** in **1x MCL TR-FRET Assay Buffer** to 8.33 ng/µl (25 ng/reaction). Initiate reaction by adding 3 µl of diluted **Mouse MCL-1** to wells designated for the "Negative Control," "Positive Control," and "Test Inhibitor." Discard any remaining diluted Mouse MCL-1 protein after use.

9) Incubate at room temperature for 1 hour.

10) 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.

$$\%\,Activity = \frac{FRET_S - FRET_{neg}}{FRET_P - FRET_{neg}} \times 100\%$$

Where $FRET_s$ = Sample FRET, $FRET_{Neg}$ = Negative control FRET, and $FRET_P$ = Positive control FRET.

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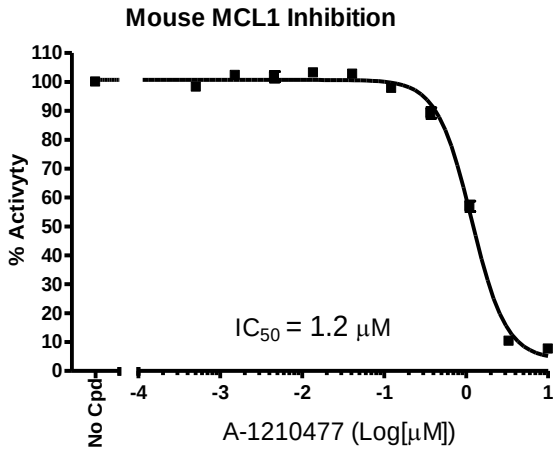
EXAMPLE OF ASSAY RESULTS:



Inhibition of Mouse MCL-1 by A-1210477, measured using the Mouse *MCL-1 TR-FRET Assay Kit*, BPS Bioscience #79928. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product	Catalog #	Size
Mouse MCL1, His-Tag	100637	
MCL1, His-Tag	40742	100 µg
Bcl-2, His-tag	50272	100 µg
Bcl-xL, His-tag	50273	100 µg
Caspase-3	80500	50 µg
Caspase-6	80113	50 µg
Caspase-7	70000	50 µg
Caspase-8	80114	50 µg
Caspase-9	80115	50 µg
Caspase-3 Homogeneous Assay Kit	80700	96 rxns.
Caspase-6 Homogeneous Assay Kit	80703	96 rxns.
Caspase-7 Homogeneous Assay Kit	80701	96 rxns.
Caspase-8 Homogeneous Assay Kit	80704	96 rxns.
NSC-632839	27709	10 mg
TW-37	27775	50 mg
(S)-HDAC-42	27208	1 mg
b-AP15 (NSC-687852)	27701	25 mg
Caspase-3/7 Inhibitor I	27741	10 mg

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

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