Data Sheet
MCL-1 TR-FRET Assay Kit
Catalog #AMS.79506
Size: 384 reactions

DESCRIPTION: The MCL-1 TR-FRET Assay Kit is designed to measure the inhibition of MCL-1 binding to its ligand in a homogeneous 384 reaction format. This 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; a sample containing terbium-labeled donor, dye-labeled acceptor, MCL-1, peptide ligand, and an inhibitor is incubated for 2 hours. Then, the fluorescence intensity is measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
AMS.40742	MCL-1	10 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
AMS.79507	MCL-1 Peptide Ligand	400 rxns	-80°C	
AMS.30017	Anti-His Tb-labeled donor	10 µl	-20°C	
	Dye-labeled acceptor	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.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

1) Dilute one part **3x MCL TR-FRET Assay Buffer** with 2 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** 200-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
MCL-1 (3.34 ng/µl)	3 µl	3 µl	3 µl
Total	**20 µl**	**20 µl**	**20 µl**

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

5) Resuspend **MCL-1 Peptide Ligand** in 320 µl of **1x MCL TR-FRET Assay Buffer**. Aliquot **MCL-1 Peptide Ligand** into single-use aliquots. Store remaining undiluted ligand at −80°C immediately. *Note: Ligand is sensitive to freeze/thaw cycles. Do not re-use thawed aliquots.*

6) Dilute **MCL-1 Peptide Ligand in step 5** 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 in step 5). Add 5 µl of this 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."

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

8) Dilute **MCL-1** in **1x MCL TR-FRET Assay Buffer** to 3.34 ng/µl (10 ng/reaction). Initiate reaction by adding 3 µl of diluted **MCL-1** to wells designated for the "Negative Control," "Positive Control," and "Test Inhibitor." Discard any remaining diluted MCL-1 protein after use.

9) Incubate at room temperature for 2 hours.

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:

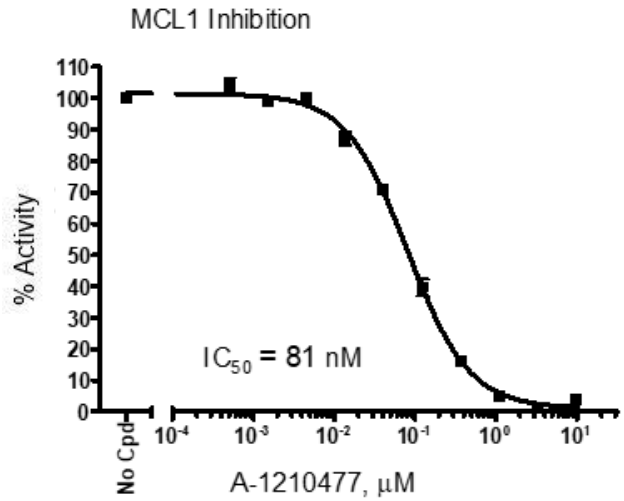
Two sequential measurements should be conducted. Tb-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

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.

EXAMPLE OF ASSAY RESULTS:



Inhibition of MCL-1 by A-1210477, measured using the *MCL-1 TR-FRET Assay Kit*, #AMS.79506. *Data shown is lot-specific.*

RELATED PRODUCTS:

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

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

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