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Data Sheet
BCL-2 TR-FRET Assay Kit
Catalog # 50222
Size: 384 reactions

DESCRIPTION: The BCL-2 TR-FRET Assay Kit is designed to measure the inhibition of BCL-2 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, BCL-2, peptide ligand, and an inhibitor is incubated for 180 minutes. Then, the fluorescence intensity is measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
50272	BCL-2	10 µg	-80°C	(Avoid freeze/thaw cycles!)
30057	BCL-2 Peptide Ligand	50 µl	-80°C	
30017	Anti-His Tb-labeled donor	2 x 10 µl	-20°C	
	Dye-labeled acceptor	2 x 10 µl	-20°C	
30059	3x BCL 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. Filippakopoulos, P., *et al.*, *Cell* 2012; **149**:214.

**Since January of 2018, this kit has been optimized for better performance. The previous version of the BCL-2 TR-FRET Assay Kit, Cat. #50222, can still be purchased upon special request.*

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

- 1) Dilute one part **3x BCL TR-FRET Assay Buffer** with 2 parts distilled water (3-fold dilution) to make **1x BCL 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 BCL 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	–
BCL TR-FRET Assay Buffer	5 µl	–	–
BCL-2 Peptide Ligand	–	5 µl	5 µl
BCL-2 (1 ng/µl)	3 µl	3 µl	3 µl
Total	20 µl	20 µl	20 µl

- 5) Thaw **BCL-2 Peptide Ligand** on ice. Upon first thaw, briefly spin tube to recover the full contents. Aliquot each ligand into single-use aliquots. Store remaining undiluted ligand at -80°C immediately. *Note: Ligand is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots.*
- 6) Dilute **BCL-2 Peptide Ligand** 40-fold in **1x BCL TR-FRET Assay Buffer**. Add 5 µL of diluted **BCL-2 Peptide Ligand** to each well designated as “Positive Control” and “Test Inhibitor.” Add 5 µL of **1x BCL TR-FRET Assay Buffer** to the wells labeled as “Negative Control.”

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- 7) Thaw **BCL-2** protein on ice. Upon first thaw, briefly spin tube containing protein to recover the full contents of the tube. Aliquot **BCL-2** protein into single-use aliquots. Store remaining undiluted aliquots at -80°C immediately. *Note: BCL-2 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*
- 8) Dilute **BCL-2** in **1x BCL TR-FRET Assay Buffer** to 1 ng/ μl (3 ng/reaction). Initiate reaction by adding 3 μl of diluted **BCL-2** to wells designated for the "Negative Control," "Positive Control," and "Test Inhibitor." Discard any remaining diluted BCL-2 protein after use.
- 9) Incubate at room temperature for 3 hours.
- 10) Read the fluorescent intensity in a microtiter-plate reader capable of TR-FRET.

Instrument Settings

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

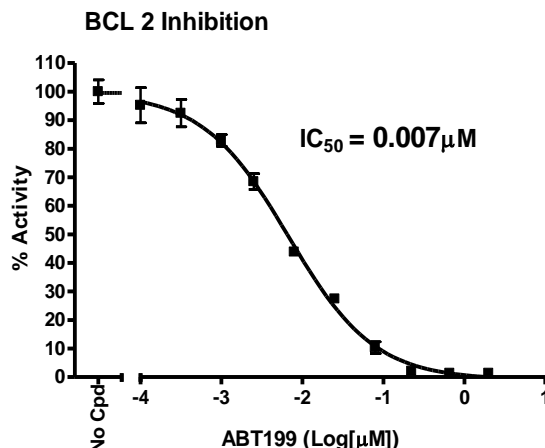
$$\% \text{ Activity} = \frac{FRET_s - FRET_{neg}}{FRET_p - FRET_{neg}} \times 100\%$$

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

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EXAMPLE OF ASSAY RESULTS:



Inhibition of BCL-2 by ABT 199, measured using the *BCL-2 TR-FRET Assay Kit*, BPS Bioscience #50222. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

RELATED PRODUCTS:

<u>Product</u>	<u>Catalog #</u>	<u>Size</u>
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
Obatoclax	27044	5 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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