Description

The BCL-XL TR-FRET Assay Kit is designed to measure the inhibition of BCL-XL (B-cell lymphoma-extra large) binding to its ligand in a Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) homogeneous 96 reaction format. This FRET-based assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications. This kit contains enough recombinant BCL-XL, BCL-XL peptide ligand, assay buffer, Anti-His Terbium-Labeled Donor and Dye-labeled Streptavidin Acceptor for 100 reactions.

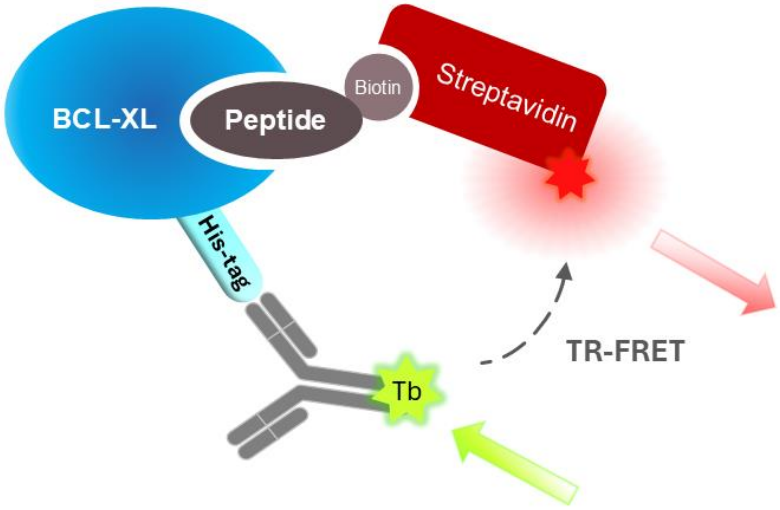

Figure 1. Illustration of the BCL-XL TR-FRET Assay Kit principle.

A sample containing terbium-labeled donor, dye-labeled acceptor, BCL-XL, peptide ligand, and an inhibitor is incubated for 180 minutes. The Anti-His Terbium-Labeled Donor binds to His-tagged BCL-XL. The BCL-XL peptide ligand is labeled with biotin, which allows the Dye-labeled Streptavidin Acceptor to bind to the BCL-XL peptide ligand. This results in energy transfer upon terbium excitation from the terbium to the acceptor. The fluorescent intensity is measured using a fluorescence plate reader capable of TR-FRET reading and an increase in 620-665 nm corresponds directly to the interaction of BCL-XL with the BCL-XL peptide ligand.

Background

BCL-XL (B-cell lymphoma-extra large), also called BCL2L1, is a member of the BCL-2 family of proteins and is involved in regulating apoptosis. BCL-XL is part of the group of BCL-2 proteins considered pro-survival proteins, as when bound to their effector proteins they inhibit apoptosis. BCL-XL plays a role in the permeability of mitochondrial membrane, allowing the release of cytochrome C. In addition to its role in apoptosis, it is also involved in neural growth, synaptic plasticity, and neuroprotection. As their name indicates, they are found at abnormal levels in B-cell lymphoma and may contribute to the progression of the disease. BCL-XL overexpression is found in about 80% of lymphomas, and it is thus an attractive target in cancer therapy. Recently it has been identified as being a player in autoimmune disorders and ageing, by controlling the apoptosis rate of immune cells, fibroblasts and other cell types. Several therapeutic approaches have been explored to target BCL-XL, ranging from small inhibitors, such as navitoclax, to PROTACs (proteolysis targeting chimeras). PROTAC 753B, a PROTAC targeting BCL-XL/BCL2 to VHL (von Hippel-Lindau), has been shown to increase the effects of chemotherapy, while avoiding off-target effect on platelets, as these do not express VHL. Advances in small inhibitors are also ongoing and promise to bring benefit to oncologic patients.

Application(s)

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
50273	BCL-XL, His-Tag*	2.1 µg	-80°C
30058	BCL-XL Peptide Ligand, Biotin-Labeled	25 µl	-80°C
	Anti-His Terbium-Labeled Donor	10 µl	-20°C
	Dye-labeled Streptavidin Acceptor	10 µl	-20°C
30059	3x BCL TR-FRET Assay Buffer	4 ml	-20°C
79696	White, nonbinding, plate	1	Room Temp

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- Adjustable micropipettor and sterile tips
- Rotating or rocker platform
- Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (λex=340 nm and detection at λem= 620-665 nm)

Storage Conditions

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

Safety

This product is for research purposes only and not for human or therapeutic use. Overall, this product should be considered hazardous and harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

- The final concentration of DMSO in the assay should not exceed 1%.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound on the assay results.

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- The assay should include "Blank", "Positive Control," and "Test Inhibitor" conditions.
- It is recommended all controls are run side by side as they may be necessary for result calculation.
- We recommend using A-1155463 (#82803) as an internal control for the assay. If not running a dose response curve for the control inhibitor, run at 0.1X, 1X, and 10X the IC_{50} value shown in the validation data below.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

1. Dilute **3x BCL TR-FRET Assay Buffer** 3-fold with distilled water. This makes **1x BCL TR-FRET Assay Buffer**.

   Note: The remaining 3x BCL TR-FRET Assay Buffer can be aliquoted and stored at -20°C.

2. Dilute **Anti-His Terbium-Labeled Donor** 100-fold with **1x BCL TR-FRET Assay Buffer** (10 µl/well).

3. Dilute **Dye-Labeled Streptavidin Acceptor** 100-fold with **1x BCL TR-FRET Assay Buffer** (10 µl/well).

   Note: The remaining Anti-His Terbium-Labeled Donor and Dye-Labeled Streptavidin Acceptor can be aliquoted and stored at -20°C (minimum volume of 5 µl/aliquot).

4. Thaw **BCL-XL Peptide Ligand** on ice. Briefly spin the tube to recover its full content.

5. Thaw **BCL-XL protein** on ice. Briefly spin the tube to recover its full content.

6. Add 10 µl of diluted **Anti-His Tb-Labeled Donor** to all wells.

7. Add 10 µl of diluted **Dye-labeled Acceptor** to all wells. Protect the plate from direct exposure to light.

8. Prepare the Test inhibitor **(4 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 40 µl.

   8.1 If the Test inhibitor is water-soluble, prepare serial dilutions in 1x BCL TR-FRET Assay Buffer, 10-fold more concentrated than the desired final concentrations.

   For positive and negative controls, use 1x BCL TR-FRET Assay (Diluent Solution).

   OR

   8.2 If the Test inhibitor is soluble in DMSO, prepare a solution at 100-fold the highest desired concentration in 100% DMSO, then dilute the compound 10-fold in 1x BCL TR-FRET Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

Using 1x BCL TR-FRET Assay Buffer in 10% DMSO, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x BCL TR-FRET Assay Buffer (vol/vol) so that wells contain the same amount of DMSO (**Diluent Solution**).

Note: The final concentration of DMSO in the assay should not exceed 1%.

9. Add 4 µl of diluted Test Inhibitor to each well designated "Test Inhibitor".

10. Add 4 µl of Diluent Solution to the wells labeled as "Blank" and "Positive Control".

11. Dilute **BCL-XL protein** in 1x BCL TR-FRET Assay Buffer to 3.5 ng/µl (6 µl/well).

12. Add 6 µl of **diluted BCL-XL protein** to all wells.

    Note: If needed, cover the plate with aluminum foil and pre-incubate the plate for 30 minutes (up to 1 hour) at Room Temperature (RT) with gentle agitation. Protect from light.

13. Dilute the **BCL-XL Peptide Ligand** 40-fold with **1x BCL TR-FRET Assay Buffer** (10 µl/well).

14. Initiate the reaction by adding 10 µl of diluted **BCL-XL Peptide Ligand** to each well designated as "Positive Control" and "Test Inhibitor".

15. Add 10 µl of 1x BCL TR-FRET Assay Buffer to the wells labeled as "Blank".

16. Tap gently to ensure that liquid is evenly distributed at the bottom of each well.

17. Cover the plate with aluminum foil and incubate at RT for 3 hours.

Component	Blank	Positive Control	Test Inhibitor
Diluted Anti-His Tb-Labeled Donor	10 µl	10 µl	10 µl
Diluted Dye-Labeled Acceptor	10 µl	10 µl	10 µl
Test Inhibitor	-	-	4 µl
Diluent Solution	4 µl	4 µl	-
Diluted BCL-XL protein (3.5 ng/µl)	6 µl	6 µl	6 µl
1x BCL TR-FRET Assay Buffer	10 µl	-	-
Diluted BCL-XL Peptide Ligand	-	10 µl	10 µl
Total	**40 µl**	**40 µl**	**40 µl**

18. Read the fluorescence intensity in a microtiter-plate reader capable of TR-FRET.

Instrument Settings

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

Reading Mode	Time Resolved
Excitation Wavelength	340 (20 nm bandwidth)
Emission Wavelength	620 (10 nm bandwidth)
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	340 (20 nm bandwidth)
Emission Wavelength	665 (10 nm bandwidth)
Lag Time	60 µs
Integration Time	500 µs

CALCULATING RESULTS

Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

$$FRET = \frac{S_{665}}{S_{620}}$$

When percentage activity is calculated, the FRET value from the Blank (it is expected that Blank and Negative Control have a similar values) can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

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

$FRET_S$ =FRET value for samples of Test Inhibitor, $FRET_{blank}$ = FRET value for the Blank, and $FRET_p$ = FRET value for the Positive Control (no inhibitor).

Example of Assay Results

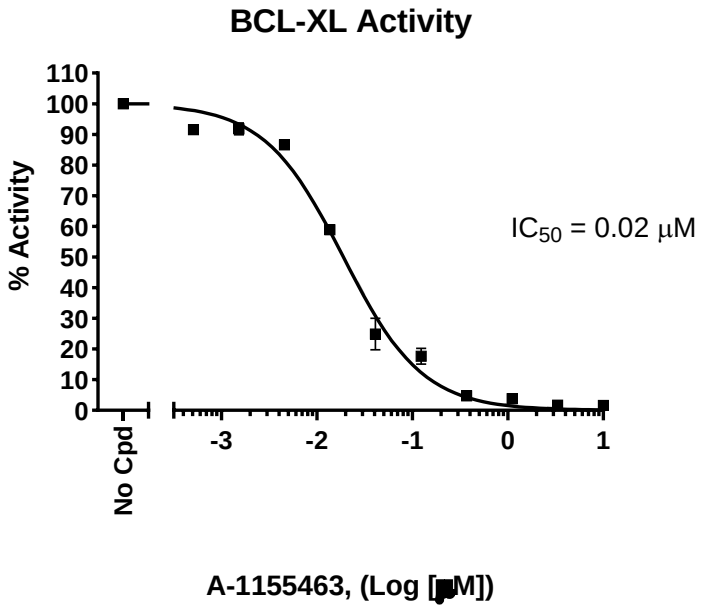

Figure 2: Inhibition of BCL-XL binding to its peptide ligand by A-1155463.
BCL-XL binding to BCL-XL Peptide Ligand was measured in the presence of increasing concentrations of A-1155463 (#82803). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (binding activity in the absence of inhibitor, set at 100%).

Data are representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References
Morales-Martinez M. and Vega M., 2022 *Int J Mol Sci* 23(4) :2193.
Li M., *et al.*, 2020 *Pharmacological Research* 151 :104547.
Jia Y., *et al.*, 2023 *Haematologica* 108(10): 2626-2638.
Lakhani N, *et al.*, 2024 *Clin Cancer Res* 30 (3) : 506-521.

Related Products

Products	*Catalog #*	*Size*
BCL-XL, His-Tag	50273	100 µg
BCL2A1 TR-FRET Assay Kit	79601	384 reactions
BCL-XL TR-FRET Assay Kit	50223	384 reactions
NSC-632839	27709	10 mg
TW-37	27775	50 mg
Obatoclax	27044	5 mg

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

Version 051225