SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

The BCL-2 TR-FRET Assay Kit is designed to measure the inhibition of BCL-2 (B-cell lymphoma 2) binding to its ligand in a 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-2, BCL-2 peptide ligand, assay buffer, Anti-His Terbium-Labeled Donor, and Dye-labeled Streptavidin Acceptor for 96 reactions.

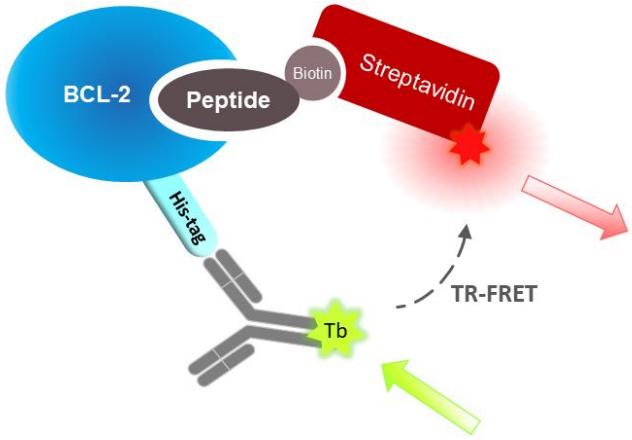

Figure 1. Illustration of the BCL-2 TR-FRET Assay Kit principle.

A sample containing terbium-labeled donor, dye-labeled acceptor, BCL-2, peptide ligand, and an inhibitor is incubated for 180 minutes. The Donor Terbium-labeled anti-His antibody binds to His-tagged BCL-2. The BCL-2 peptide ligand is labeled with biotin, which allows the dye-labeled streptavidin acceptor to bind to the BCL-2 peptide ligand. The TR-FRET signal is generated by proximity induced upon interaction of BCL-2 with the BCL-2 peptide ligand. Then, the fluorescence intensity is measured using a fluorescence reader capable of TR-FRET measurements. The signal generated is proportional to BCL-2 activity.

Background

B-cell lymphoma 2 (BCL-2) is a member of the BCL family that regulates apoptosis through pro-apoptotic or anti-apoptotic signals. BCL-2 is normally localized at the outer membrane of mitochondria and blocks pro-apoptotic signals, promoting cell survival. Genetic alteration of BCL-2 has been observed in various types of cancer, including lymphoma, leukemia, melanoma, as well as breast and prostate cancer. The protein is also involved in cancer cell resistance to treatment due to its pro-survival functions. ABT-199, also known as Venetoclax, is a BH3-mimetic that specifically blocks the function of BCL-2 and was the first BCL-2 targeted agent to receive FDA approval (in 2016). It is now approved for the treatment of chronic lymphocytic leukemia and small lymphocytic lymphoma.

Application(s)

Screen small molecule inhibitors in drug discovery and high-throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
50272	BCL-2, His-Tag*	1 µg	-80°C
30057	BCL-2 Peptide Ligand, Biotin-Labeled	25 µl	-80°C
30017	Anti-His Terbium-Labeled Donor	10 µl	-20°C
	Dye-labeled Streptavidin Acceptor	10 µl	-20°C
30059	BCL TR-FRET Assay Buffer	4 ml	-20°C
79696	White, nonbinding, low volume microtiter plate	1	Room Temperature

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

Materials Required but Not Supplied

- Fluorescent 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.

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.

Assay Protocol

- The assay should include “Negative Control”, “Positive Control” and “Test Compound” conditions.
- We recommend using ABT-199 as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1 x, 1 x and 10 x 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).

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

   Note: The remaining 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.

4. Thaw **BCL-2 Peptide Ligand** on ice. Briefly spin the tube to recover its full content.

5. Dilute the **BCL-2 Peptide Ligand** 40-fold with **1x BCL TR-FRET Assay Buffer** (10 µl/well).

6. Thaw **BCL-2 protein** on ice. Briefly spin the tube to recover its full content.

7. Add 10 µl of diluted **Anti-His Tb-Labeled Donor** to the "Test Inhibitor", "Negative Control" and "Positive Control" wells.

8. Add 10 µl of diluted **Dye-labeled acceptor** to each well designated "Test Inhibitor," "Negative Control," and "Positive Control."

9. Prepare the Test compound **(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.

   9.1. If the Test compound is water-soluble, prepare serial dilutions in 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

   9.2 If the Test compound 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 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 all wells contain the same amount of DMSO (**Diluent Solution**).

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

10. Add 4 µl of diluted Test Compound to each well designated "Test Compound".

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

12. Add 10 µl of diluted BCL-2 Peptide Ligand to each well designated as "Positive Control" and "Test Inhibitor".

13. Add 10 µl of 1x BCL TR-FRET Assay Buffer to the wells labeled as "Negative Control".

14. Dilute **BCL-2 protein** in 1x BCL TR-FRET Assay Buffer to 1 ng/µl (6 µl/well).

15. Initiate the reaction by adding 6 µl of **diluted BCL-2 protein** to wells designated "Negative Control", "Positive Control" and "Test Compound".

Component	Negative Control	Positive Control	Test Compound
Anti-His Tb-Labeled Donor	10 µl	10 µl	10 µl
Dye-Labeled Acceptor	10 µl	10 µl	10 µl
Test Compound	-	-	4 µl
Diluent Solution	4 µl	4 µl	-
1x BCL TR-FRET Assay Buffer	10 µl	-	-
Diluted BCL-2 Peptide Ligand	-	10 µl	10 µl
Diluted BCL-2 protein (1 ng/µl)	6 µl	6 µl	6 µl
Total	**40 µl**	**40 µl**	**40 µl**

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

17. Incubate at room temperature for 3 hours.

18. Read the fluorescence intensity in a microtiter-plate reader capable of TR-FRET. Two sequential measurements should be conducted. Tb-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm.

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

Data Analysis

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.

Example of Assay Results

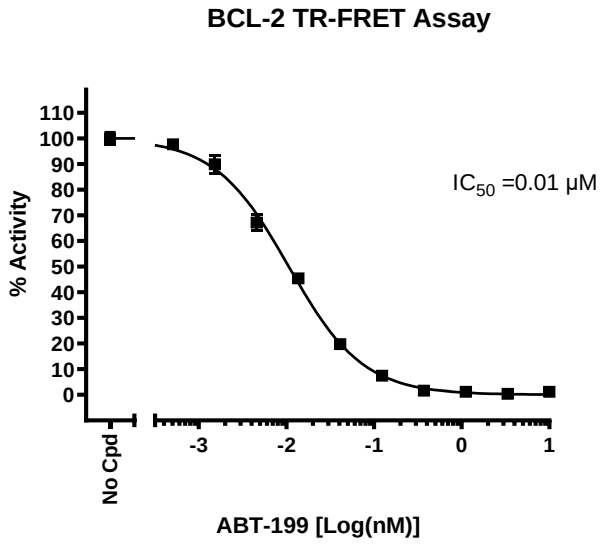

Figure 2: Inhibition of BCL-2 binding to its peptide ligand by ABT-199.
BCL-2 binding to BLC-2 Peptide Ligand was measured in the presence of increasing concentrations of ABT-199 (Adooq Bioscience #A12500). The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (kinase activity in the absence of inhibitor, set at 100%).

Data are representative. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

References

Filippakopoulos P., *et al.*, 2012, *Cell*, 149: 214.

Related Products

Products	*Catalog #*	*Size*
BCL-XL, His-Tag	50273	100 µg
BCL2L2, His-Tag	100091	100 µg
BCL2L10, His-Tag	100080	100 µg
BCL2A1, His-Tag	100070	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 110623