Description

The DCAF1 Binding Assay Kit is a fluorescence polarization (FP), homogeneous, 96-well assay kit designed for the screening and profiling of molecules that inhibit the binding of the DCAF1 (DDB1 and CUL4 Associated Factor 1) Complex to a high affinity DCAF1 fluorescent probe. The kit contains enough purified recombinant DCAF1/Rbx1 (M5I)/DDB1/CUL4A Complex, fluorescent probe and assay buffer for 100 reactions.

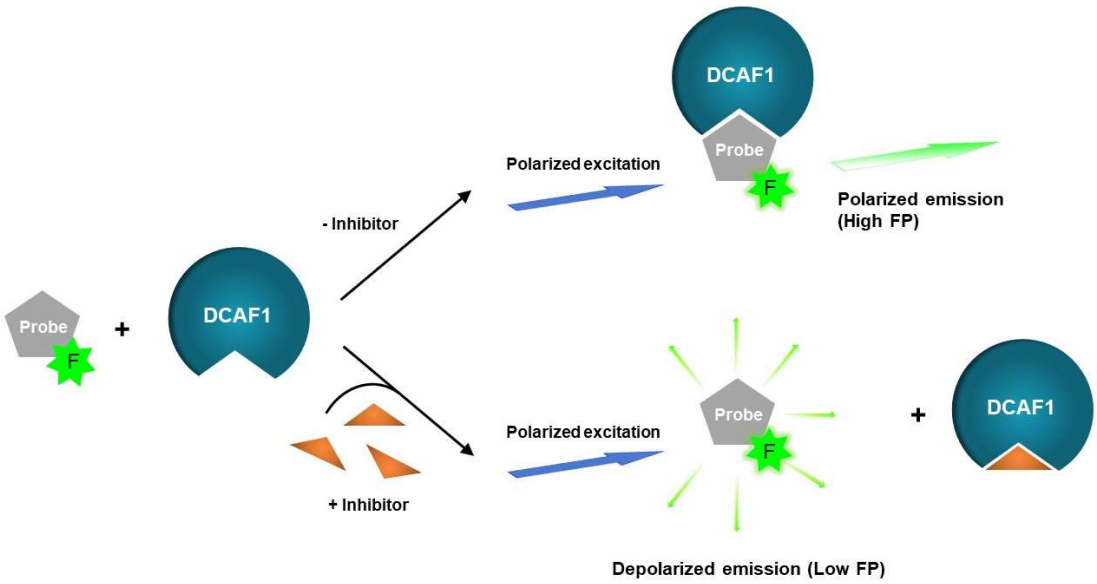

Figure 1: Illustration of the DCAF1 Binding Assay Kit principle.

The assay uses a DCAF1 fluorescent probe. This is a very small molecule that rotates fast (low FP). This probe is incubated with the DCAF1 complex leading to the formation of a large complex, with restricted movement (high FP). FP is proportional to binding of DCAF1 to the fluorescent probe.

This assay requires a fluorescent microplate reader capable of measuring fluorescence polarization (FP) and equipped with the required parts to read the FP signal. For more information FP technology, visit our Tech Note: FP, assay principles and applications.

Background

DCAF1 (DDB1 and CUL4 Associated Factor 1), also known as VprBP (Vpr binding protein), is a WD40 repeat domain-containing E3 ligase of the CRL4 (cullin RING ligase 4) subfamily of proteins. It functions as the substrate recognition unit in E3 ligase complexes, such as the EED (embryonic ectoderm development)-DDB1 (DNA damage-binding protein)-VprBP (or EDVP) and the $CRL4^{DCAF1}$ complex. The $CRL4^{DCAF1}$ complex is composed of CUL4A (cullin-4A), DDB1 and DCAF1. DCAF1 is involved in various normal physiological functions in the cell, including cell cycle regulation/progression, cell division, lipid metabolism, and miRNA biogenesis. Additionally, DCAF1 plays an E3 ligase-independent role, by acting as a kinase and phosphorylating Histone H2A. Antagonists of DCAF1 could be used toward the development of therapeutics for cancer and viral treatments. DCAF1 can also be used to create bifunctional molecules such as proteolysis-targeting chimeras to induce degradation of proteins of interest. For instance, the use of a DCAF1-BTK (Bruton's tyrosine kinase) PROTAC® resulted in degradation of BTK in cells that had acquired resistance to CRBN (cereblon)-BTK PROTACs, making DCAF1 an interesting alternative E3 ligase in cases of resistance mechanism to other commonly used PROTACs. Design and characterization of DCAF1 binders is an important step in the design of DCAF1-targeting molecular degraders.

Applications

Study and screen small molecule inhibitors that inhibit the binding of DCAF1 to the fluorescent probe for drug discovery in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
102190	DCAF1/Rbx1 (M5I)/DDB1/CUL4A Complex*	100 µg	-80°C
	10 µM BDY DCAF1 Substrate	15 µl	-80°C
	DCAF1 Assay Buffer (FP)	10 ml	-20°C
79685	Low binding, black 96-well plate	1	Room Temp.

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

Materials Required but Not Supplied

- Adjustable micropipettor and sterile tips
- Rotating or rocker platform
- Fluorescent microplate reader capable of measuring fluorescence polarization (λex=485/20 nm and detection at λem=528/20 nm)

Stability

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. This product should be considered hazardous and is 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%.
- Fluorescent compounds that have λex=485/20 nm and detection at λem=528/20 nm can interfere with the readouts.

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- The assay should include "Blank", "Reference Control" (minimum FP), "Positive Control" (maximum FP), and "Test Inhibitor" conditions.
- It is recommended to run all controls side by side as they may be necessary for result calculation.
- We recommend using DCAF1 binder 1 (MedChemExpress #HY-149934) 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. Thaw **DCAF1 Complex** on ice. Briefly spin the tube containing the enzyme to recover its full content.

2. Dilute DCAF1 Complex with DCAF1 Assay Buffer (FP) to 25 ng/µl (40 µl/well), by performing a serial dilution. For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

3. Add 40 µl of diluted DCAF1 Complex to the "Positive Control" and "Test Inhibitor" wells.

4. Add 45 µl of DCAF1 Assay Buffer (FP) to the "Blank" wells.

5. Add 40 µl of DCAF1 Assay Buffer (FP) to the "Reference Control" wells.

6. Prepare the Test Inhibitor (5 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 50 µl.

   6.1 If the Test Inhibitor is water-soluble, prepare serial dilutions 10-fold more concentrated than the desired final concentrations in DCAF1 Assay Buffer (FP).

   For the positive and negative controls, use DCAF1 Assay Buffer (FP) as Diluent Solution.

   OR

   6.2 If the Test inhibitor is soluble in DMSO, prepare the inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired concentration, then dilute the inhibitor 10-fold in DCAF1 Assay Buffer (FP) to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Use 10% DMSO in DCAF1 Assay Buffer (FP) (vol/vol) for the serial dilution to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in DCAF1 Assay Buffer (FP) (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

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

7. Add 5 µl of the inhibitor serial dilution to the "Test Inhibitor" wells.

8. Add 5 µl of the Diluent Solution to the "Blank", "Reference Control", and "Positive Control" wells.

9. Incubate at Room Temperature (RT) for 1 hour with gentle agitation.

10. Thaw **10 µM BDY DCAF1 Substrate** on ice. Briefly spin the tube containing the enzyme to recover its full content.
11. Dilute 10 µM BDY DCAF1 Substrate 66.7-fold with DCAF1 Assay Buffer (FP) (5 µl/well).

12. Add 5 µl of diluted BDY DCAF1 Substrate to the "Positive Control", "Reference Control" and "Test Inhibitor" wells.

13. Incubate at RT for 30-45 minutes with gentle agitation.

Component	Blank	Reference Control	Positive Control	Test Inhibitor
Diluted DCAF1 Complex (25 ng/µl)	-	-	40 µl	40 µl
DCAF1 Assay Buffer (FP)	45 µl	40 µl	-	-
Test Inhibitor	-	-	-	5 µl
Diluent Solution	5 µl	5 µl	5 µl	-
1 hour at RT				
Diluted BDY DCAF1 Substrate	-	5 µl	5 µl	5 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

14. Read FP in a fluorescence plate reader capable of measuring fluorescence polarization (λex = 485 ± 20 nm; λem = 528 ± 20 nm).

15. Subtract the "Blank" value from all other values.

Calculating Results

Fluorescence polarization is a measure of the amount of molecular rotation that takes place in the time between excitation and emission of the fluorescence probe. It can be determined from the measurements of perpendicular ($I_{\perp}$) and parallel ($I_{\parallel}$) fluorescence intensity values emitted by the probe relative to the direction of the polarized excitation light (Figure 2).

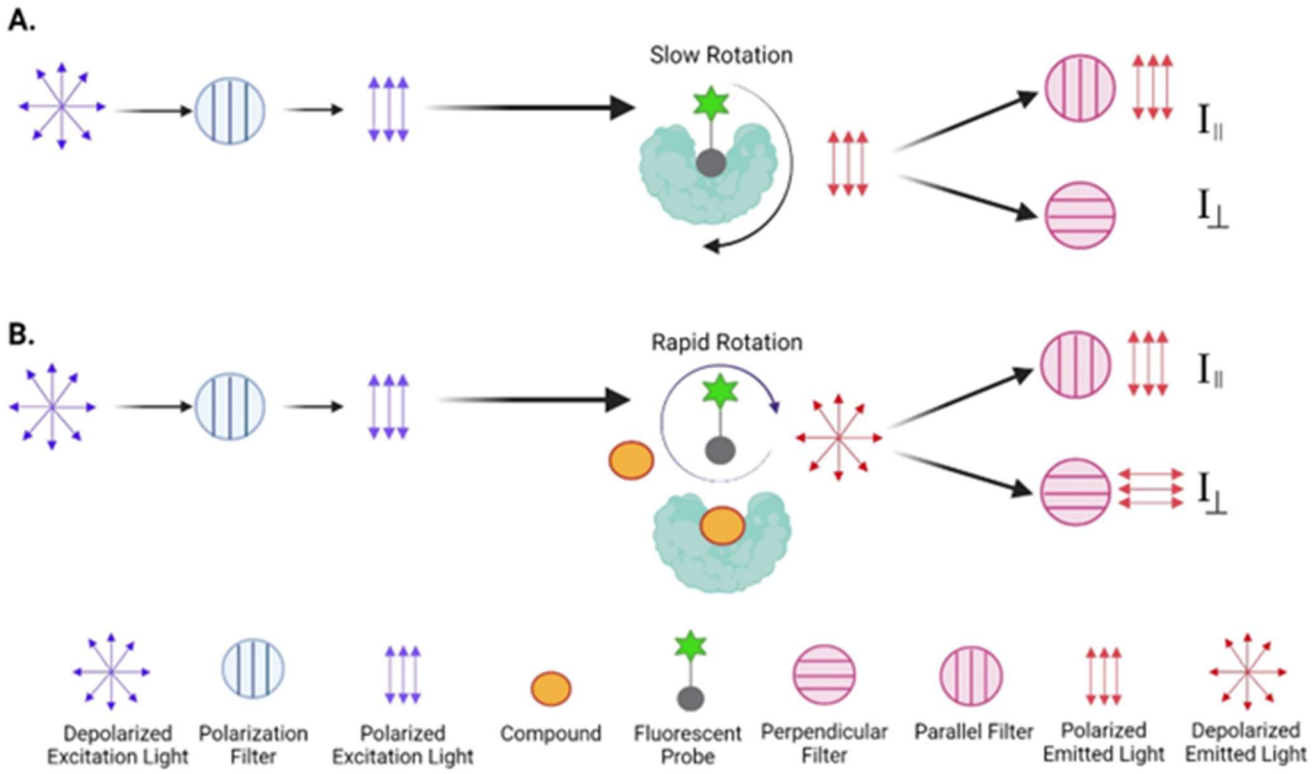

Figure 2: Fluorescence polarization principle.
A. When the fluorescently labeled probe binds to a larger protein it creates a complex of a big molecular weight that has a slow rotation ability. In this state the probe has a reduced rotational diffusion so when it is excited by polarized light, it still emits highly polarized light with a degree of polarization that is inversely proportional to the rate of molecular rotation.
B. In the presence of a compound that has affinity for the protein, the fluorescent probe remains in solution and can rotate rapidly. Unbound probe has a high rotational diffusion so when it is excited by the polarized light it emits light in orientations that can be detected by both the perpendicular and parallel filters.

Polarization is defined as the difference between the emission intensities of parallel fluorescence (I_{II}) and perpendicular fluorescence ($I_{\perp}$), divided by the total fluorescence emission intensity. The polarization value (P) being a ratio of light intensities, is a dimensionless number, often expressed in milli P units where 1 P unit = 1000 mP units. To calculate P one has to take into consideration that light is not transmitted equally well through both parallel and perpendicular channels and therefore a correction must be made. This correction factor is called the "G Factor" (G) and it is specific to the instrument used. mP can thus be calculated using the following formula:

$$mP = \left(\frac{I_{II} - G(I_{\perp})}{I_{II} + G(I_{\perp})}\right) x\ 1000$$

Modern instruments usually have the G factor pre-calculated and can automatically calculate fluorescence polarization for your experiments. If you need to determine, set up or calculate the G factor please refer to your instrument manual (the instrument manual should contain information about how to establish the G-factor) or check our FAQ section (FAQs (bpsbioscience.com).

For accurate calculations it is necessary to provide the correct plate schematic when setting up your instrument, with defined positions for the "Blank" and "Reference" (also known as Substrate Control) wells, and to ensure that the emission intensities from the "Blank" wells are subtracted from all other wells prior to further data analysis.

We encourage you to analyze raw data and if appropriate to exclude those "Blank" or "Reference" wells that show aberrant readouts prior to mP determination.

The % of Activity can be calculated as follows:

$$\%\ of\ Activity = \frac{(mP\ value\ from\ Test\ Inhibitor - mP\ value\ from\ Reference\ Control)}{(mP\ value\ from\ Positive\ Control - mP\ value\ from\ Reference\ Control)} x\ 100$$

Example of Assay Results

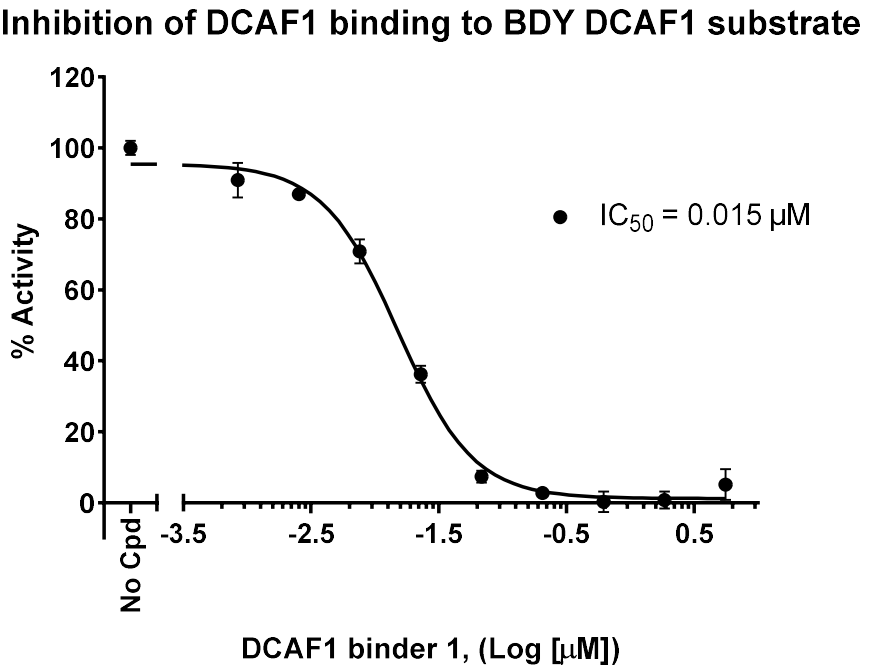

Figure 2: Inhibition of binding of DCAF1 complex to BDY DCAF1 Substrate by the inhibitor DCAF1 binder 1.
DCAF1 complex was incubated with increasing concentrations of DCAF1 binder 1 (MedChemExpress #HY-149934), in the presence of BDY DCAF1 Substrate. Fluorescence Polarization was measured using a Tecan M1000 fluorescent microplate reader. Results are expressed in % activity, in which FP in the absence of inhibitor (positive control) is set to 100%.

Data shown is representative. For lot-specific information, contact BPS Bioscience, Inc. at support@bpsbioscience.com

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For further questions, email support@bpsbioscience.com

References

Li A., *et al.,* 2023 *J Med Chem* 66 (7): 5041-5060.
Schröder M., *et al.,* 2024 *Nat Commun* 15 (1): 275.

Related Products

Products	*Catalog #*	*Size*
VHL/CUL2/ELOB/ELOC/RBX1 Complex Recombinant	100373	10 µg/50 µg
Cereblon/DDB1/Cul4A/Rbx1 Complex Recombinant	100329	10 µg/50 µg
Cereblon Ubiquitination Homogeneous Assay Kit	79881	384 reactions
ELOB/ELOC/VHL Complex Recombinant	100361	10 µg/50 µg
PROTAC® Optimization Kit for BET Bromodomain-Cereblon Binding	79770	384 reactions
PROTAC® Optimization Kit for BET Bromodomain-Von Hippel Lindau (VHL) Binding	79790	384 reactions

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