User Manual

SPRINTer™ eXpress Endogenous Protein Turnover Biosensor Assay

For Detection of Drug-Mediated Modulation of Endogenous Protein Levels

Document Number 70-451 Revision 0

Table of Contents

Table of Figures

Overview

SPRINTer™ Endogenous Protein Turnover Biosensor Assays provide a cell-based approach to monitor drug-induced protein turnover of a specific endogenous target. Genome engineering or editing techniques, such as CRISPR/Cas9, are used to introduce a small tag into the endogenous locus of interest. This allows for the quantitation of changes in target protein levels when cells are treated with therapeutic agents such as targeted protein degraders.

Assay Principle

The SPRINTer assay utilizes the established Enzyme Fragment Complementation (EFC) technology. EFC uses a split β-galactosidase (β-gal) enzyme, which consists of the Enzyme Donor (ED) and the Enzyme Acceptor (EA) fragments. Independently, these fragments have no β-gal enzymatic activity. However, when the 2 fragments are brought in close proximity, (e.g., as a result of protein-protein interaction) they are forced to complement and form an active β-gal enzyme that can then hydrolyze a chemiluminescent substrate to produce a light signal. This is represented/illustrated in Figure 1 below.

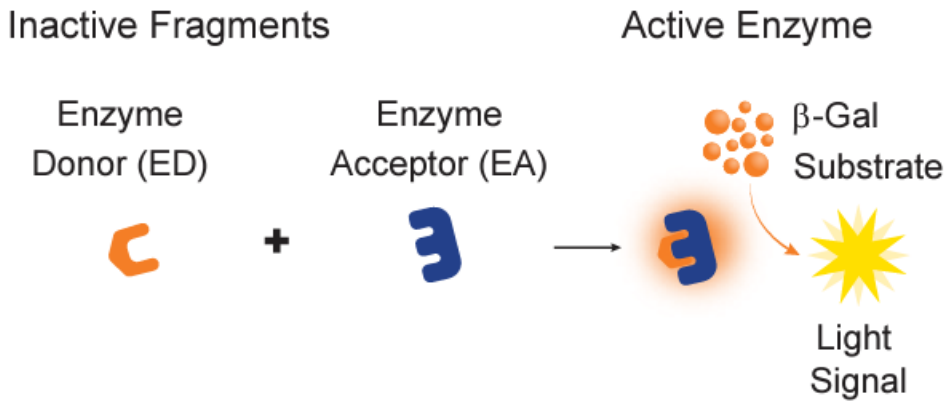
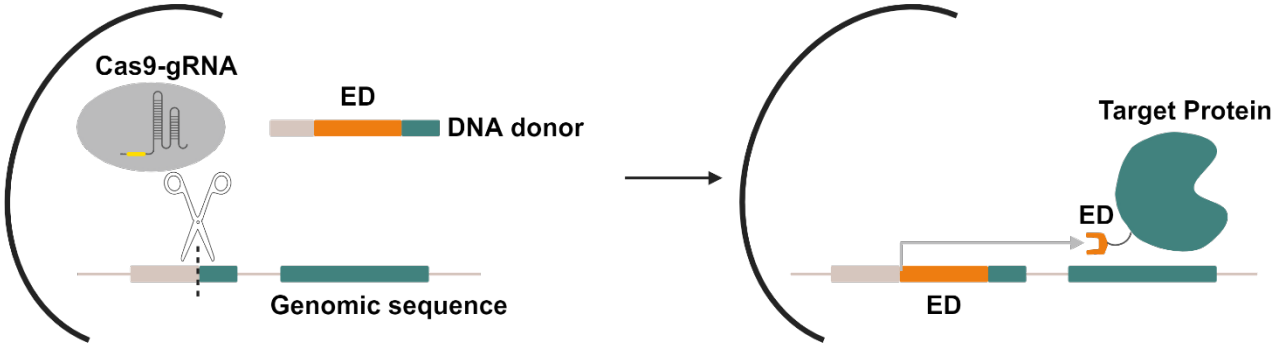


Figure 1. Enzyme Fragment Complementation (EFC) technology

The SPRINTer™ Endogenous Protein Turnover Biosensor Cell Lines have been engineered to introduce the small 42 amino acid fragment of β-gal, ED, into the endogenous locus of the desired target gene (e.g., BRD4, c-MYC) in an appropriate cell background (e.g., the colorectal carcinoma model HCT-116 line or the chronic myeloid leukemia model K-562 line). Expression of the target gene from its native promoter results in the production of an ED-tagged target protein at endogenous level, as shown in Figure 2 below.



Created with BioRender.com

Figure 2. CRISPR-mediated creation of ED-fusion protein: A schematic illustration of CRISPR-mediated knock-in of ED tag into a target locus to create an ED-fusion protein for EFC assay. Cas9- gRNA complex and a donor DNA encoding the ED fragment are delivered into cells. Cell clones with robust EFC signal are isolated and optimized for protein turnover assay.

Addition of exogenous Enzyme Acceptor (EA) and buffer lyses the cell and forces complementation of the ED and EA enzyme fragments. This results in the formation of a functional β-gal enzyme that hydrolyzes substrate to generate a chemiluminescent signal, allowing quantitation of drug-induced changes in ED-target protein levels. Treatment of these engineered biosensor cells with therapeutics that promote turnover of the target protein (such as PROTACs-mediated protein degradation) results in a decrease in the chemiluminescent signal, as illustrated in Figure 3 below.

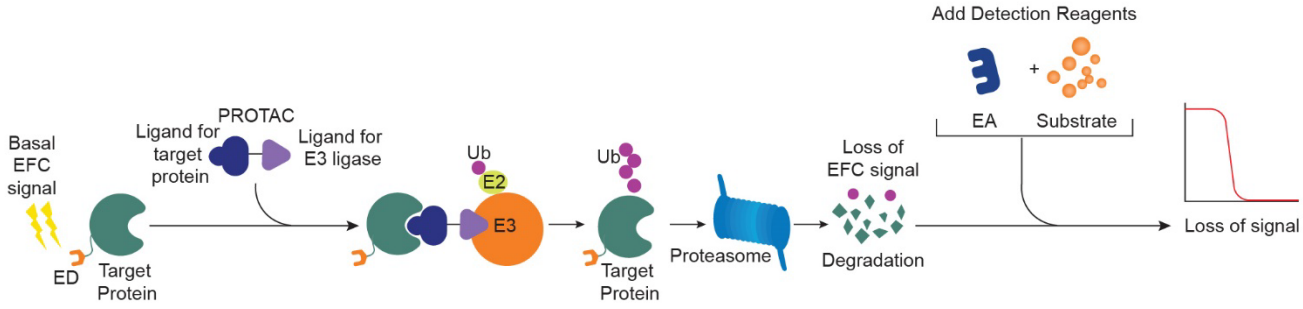


Figure 3. SPRINTer™ Biosensor Cell Lines Assay Principle: Example of an application for the SPRINTer Endogenous Protein Turnover Biosensor Cell Line: measurement of PROTAC-mediated endogenous protein turnover. When no PROTAC is present, the ED-tagged target protein is maintained at endogenous levels. Upon application of exogenous EA contained in the detection kit, substrate is hydrolyzed to produce a signal proportionate to the amount of ED-tagged target protein present. Upon addition, the heterobifunctional PROTAC molecule facilitates ubiquitination of the ED-tagged protein by bringing it into proximity with a specific E3 ligase. As a result, the ED-tagged protein undergoes degradation. A decrease in the chemiluminescent signal proportional to the amount of ED-tagged target protein that has been degraded is observed when the complementary enzyme fragment, EA, is added with the detection reagents.

Assay Workflow

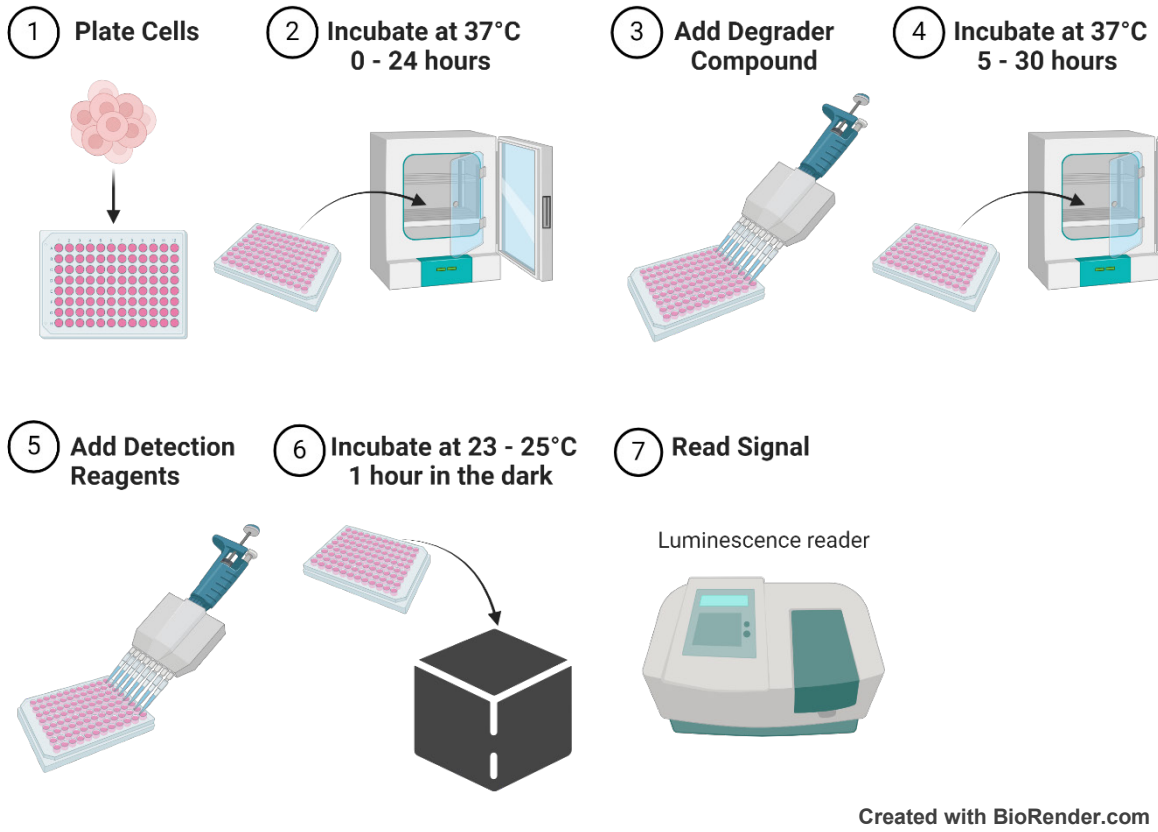


Figure 4. Assay Workflow

Please read the entire protocol completely before running the assay. Successful results depend on performing these steps correctly. The Assay Procedure sections, and Quick Start Guides contain detailed information about how to run the assays.

Assays should be run using thawed and plated cryopreserved SPRINTer™ eXpress cells from cryovials. Following treatment of the cells with compound, endogenous protein turnover is detected by adding a working solution of chemiluminescent PathHunter® PL/PK Detection reagents using a simple, add-and-read protocol.

Monitor drug-induced endogenous protein turnover activity with these SPRINTer™ eXpress Endogenous Protein Turnover Assay steps (Figure 4).

1. Thaw and Plate cells
2. Incubate at 37°C for 0-24 hours
3. Dilute and add compounds
4. Incubate at 37°C for 5-30 hours
5. Add detection reagents
6. Incubate at room temperature for 1 hour
7. Read samples

Materials Provided

List of Components	2-Plate Kit	10-Plate Kit
SPRINTer™ eXpress Endogenous Protein Turnover Biosensor Cells	2 vials	10 vials
Assay Complete™ Cell Plating Reagent*	1 x 100 mL	2 x 100 mL
PathHunter® PL/PK Detection Kit: - EA Reagent	20 mL	20 mL
- Lysis Buffer	20 L	20 mL
- Substrate Reagent	80 mL	80 mL
- Positive Control Peptide (ED)	200 µL	200 µL
96-well white-walled clear-bottom tissue culture treated plate	2 plates	10 plates

*** Cell Plating Reagent is recommended for thawing and plating the cells and for compound dilution. Please refer to the target-specific datasheet for additional details.**

Table 1. Materials Provided

Storage Conditions

SPRINTer™ eXpress Cells

Cells are shipped on dry ice and should arrive in a frozen state. To ensure maximum cell viability, store the vials of cells in the vapor phase of liquid nitrogen as soon as possible upon receipt. Please contact technical support immediately, if the cells received were already thawed.

- Short-term (24 hours or less): Store vials at -80°C immediately upon arrival. (DO NOT store at -80°C for longer than 24 hours).
- Long-term (greater than 24 hours): Vials should ONLY be stored in the vapor phase of liquid nitrogen.

Safety Warning: A face shield, gloves and lab coat should be worn at all times when handling frozen vials. Use tongs to remove cryovials from liquid nitrogen storage and place the vials immediately on dry ice in a covered container. Wait at least 1 minute for any liquid nitrogen that may be present inside the vial to evaporate. Do not touch the bottom of the vials at any time to avoid inadvertent thawing of the cells.

AssayComplete™ Cell Plating Reagent

Upon receipt, store at -20°C. Once thawed, the Cell Plating Reagent can be stored at 4°C for up to 4 weeks. For longer storage (up to the expiration date listed on the kit's Certificate of Analysis), the reagent should be aliquoted and stored at -20°C until needed. Avoid multiple freeze-thaw cycles. To make aliquots suitable for testing one assay plate each, 20 mL of reagent per aliquot can be dispensed and frozen down.

PathHunter® PL/PK Detection Kit

Upon receipt, store at -20°C. Once thawed, the detection reagents can be kept at 4°C for up to 4 days. For long- term storage (up to the expiration date listed on the kit's Certificate of Analysis), the reagent should be aliquoted and stored at -20°C until needed. Avoid multiple freeze-thaw cycles.

96-well Tissue Culture-Treated Plates

Upon receipt, store at room temperature.

Additional Materials Required

The following equipment and additional materials are required to perform these assays:

Material	Ordering Information
Serial Dilution Plates	discoverx.com/product-category/cell-culture-kits-reagents
Sterile disposable reagent reservoir	Thermo Fisher Scientific, Cat. No. 8094 or similar
Multimode or Luminescence plate reader	Refer to the Instrument Compatibility Chart at discoverx.com/tools-resources/instrument-compatibility/
Single and multi-channel micro-pipettes and pipette tips (10 µL-1,000 µL)	
Polypropylene tubes 50 mL and 15 mL	
Microcentrifuge tubes (1.5 mL)	
Tissue Culture Disposables (Pipettes 1 mL to 25 mL)	
Humidified tissue culture incubator (37° C and 5% CO_2)	

Table 2. Materials Required

Unpacking Cell Cryovials

Cryovials are shipped on dry ice and contain cells in AssayComplete™ Freezing Reagent (refer to the cell line-specific datasheet for the number of cells provided in the vial). Upon receipt, the vials should be transferred to the vapor phase of liquid nitrogen for storage beyond 24 hours. The following procedures are for safe storage and removal of cryovials from liquid nitrogen storage.

1. Cells must arrive in a frozen state on dry ice.

   Contact technical support immediately, if cells are thawed upon arrival.

2. Frozen cells must be transferred to either liquid nitrogen storage or a -80°C freezer immediately upon arrival. If cells will be thawed and used within 24 hours, they can be stored temporarily at -80°C. For longer storage, place vials in the vapor phase of liquid nitrogen storage.

   A face shield, gloves, and a lab coat should be worn during these procedures.

3. When removing cryovials from liquid nitrogen storage, use tongs and place immediately on dry ice in a covered container. Wait at least one minute for any liquid nitrogen inside the vial to evaporate.

   Cryovials should be stored in the vapor phase of liquid nitrogen. They are not rated for storage in the liquid phase.

4. Proceed with the thawing and propagation protocols in the following section. Refer to the cell line-specific datasheet for appropriate AssayComplete products mentioned in the protocol below.

Protocol Schematic

Tip: *Use this sheet to note your assay specific conditions. Post it on your bench to use as a quick reference guide.*

Assay Name: _____Date: _____

Product Details: _____

Quick-start Procedure: The flowchart below summarizes the assay protocol to be run in a 96-well plate.

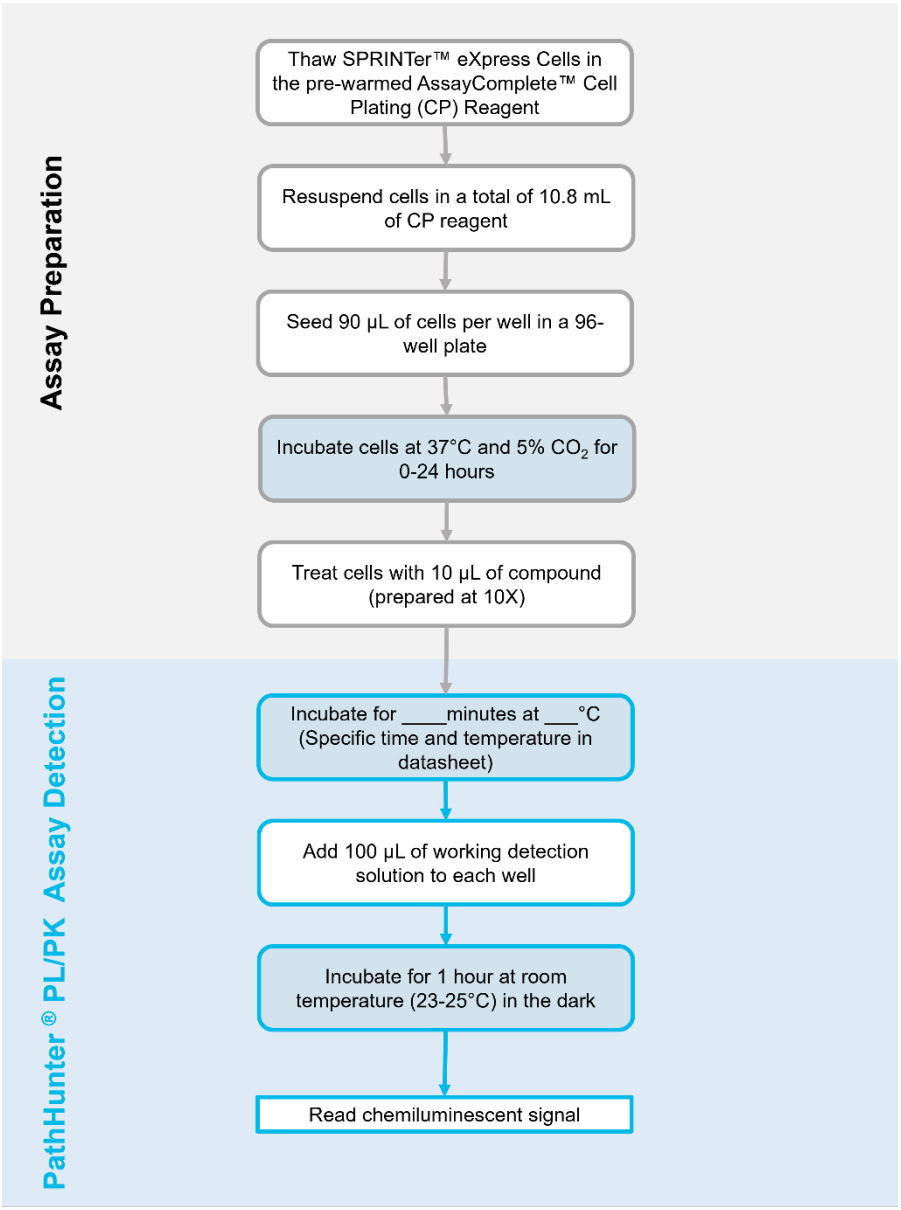


Figure 5. Quick-start Procedure

Detailed Assay Protocol (Small Molecules or Targeted Protein Degraders)

The following protocol is designed for evaluating the ability of small molecules or targeted protein degraders (e.g., molecular glues or PROTACs) to promote target protein turnover, in a 96-well format.

Refer to the table below for assay reagent volumes.

Assay Reagents (Volume per Well)	96-Well Plate	384-Well Plate**
Cell Plating Volume (µL)*	90	20
Compound (µL)	10	5
Working Detection Solution (µL)	100	25
Total Assay Volume	**200**	**50**

* **Refer to the cell line-specific datasheet for the recommended cell density.**

** **For an assay in a 384-well plate, a recommended cell number per well would be 0.5X the recommended cell number per well for a 96-well plate. Additional optimization of the cell number may be required.**

Table 3. Assay reagent volumes per well for 96-well and 384-well plates

Section I: Cell Thawing and Plating

The following protocol is for thawing and plating cryopreserved SPRINTer™ eXpress cells from cryovials.

1. Pre-warm the AssayComplete™ Cell Plating (CP) Reagent in a 37°C water bath.
2. Dispense 10.8 mL of the pre-warmed CP reagent into a 15 mL conical tube.
3. Remove the cryovials from the liquid nitrogen vapor and immediately place it in dry ice.

DO NOT use a heated water bath to thaw the vial. Hold the cryovials by the cap; DO NOT touch the sides or bottom of the vial to avoid thawing of the cell pellet. Wipe down the outside surface of the cryovial quickly with 70% ethanol, and immediately bring it into the tissue culture hood.

4. Add 0.5 mL of pre-warmed CP reagent from the 15 mL conical tube into the cryovial to thaw the cells. Slowly pipette the cell suspension up and down several times to uniformly resuspend the cells.
5. Transfer the cell suspension to the conical tube containing the remaining 10.3 mL of CP reagent. Remove any remaining suspension from the cryovial to ensure maximum recovery of all the cells.
6. Replace the cap on the conical tube and gently invert it several times to ensure that the cells are properly resuspended in the CP reagent, without creating any froth in the suspension. Immediately pour the suspension into a sterile 25 mL reagent reservoir.

7. If running the assay in a 96-well plate, transfer 90 µL of the cell suspension to each well (10,000 cells/well) of the plate, using a multichannel pipette. For recommendations on running the assay in a 384-well plate, refer to the Supplemental Information section.

8. Incubate the assay plate according to the time and conditions indicated in the target-specific datasheet. Typically, plate incubation is done at 37°C and 5% CO_2, but the incubation time is specific for each kit.

Refer to the target-specific datasheet for any variation in assay conditions.

9. Proceed to the compound preparation and addition steps.

Section II: Compound Preparation and Addition

When optimizing assay conditions, it is recommended to include a standard curve of a reference compound to verify that the assay is working optimally. For the SPRINTer™ BRD4 and c-Myc Biosensor cell lines, we recommend the PROTAC MZ1 as reference compound (Tocris, Cat. No. 6154). MZ1 is also used as an example in the protocol below.

Follow the steps below to prepare serial dilutions of the reference compound or sample in a 96-well master dilution plate by making 4-fold, 11-point serial dilutions in the appropriate AssayComplete™ Cell Plating (CP) Reagent containing the appropriate percentage of vehicle (DMSO), or the appropriate ligand dilution buffer (as specified on the ligand datasheet). This protocol should allow for each compound or sample dilution to be run in duplicate (see Figure 7, below).

Note: The reference compound or sample should be prepared at 10X the final screening concentration. To maintain constant vehicle (DMSO) concentrations for all dilutions, we recommend that serial dilutions be prepared in an appropriate dilution buffer supplemented with appropriate percentage of vehicle (< 5% DMSO recommended).

1. Prepare a compound stock solution by dissolving the dry compound in an appropriate reconstitution solution.

    a. For MZ1, top dose in the assay is 10 µM (10X is 100 µM). Add 5 µL of 2 mM MZ1 stock (in 100% DMSO) to 95 µL of appropriate AssayComplete Cell Plating Reagent.

    b. Refer to the target-specific datasheet for the recommended top screening concentration of the reference compound.

2. The steps below are for preparing 4-fold serial dilutions of the compound in an 11-point dose curve using a dilution plate. For running the assay in a 96-well plate, the concentration of each dilution should be prepared at 10X the final screening concentration. For recommendations on running the assay in a 384-well plate, refer to the Supplemental Information section.

    a. For each compound, label wells of a dilution plate numbers 1 through number 12 (Figure 6).

    b. Add 80 µL of the intermediate stock compound solution that was prepared in Step 1 to well number 12.

    c. Add 60 µL of the supplied CP Reagent to well numbers 1 through number 11.

    d. Using a clean pipette tip, transfer 20 µL of the diluted compound from well number 12 to well number 11. Mix thoroughly by pipetting up and down several times.

    e. With a clean pip pipette tip, remove 20 µL of diluted compound from well number 11 and add it to well number 10. Mix thoroughly by pipetting up and down several times.

    f. Repeat this process until Well 2 is reached, resulting in an 11-point 1:4 dilution series. No compound is transferred to Well 1 as this is the negative control well.

g. Repeat the process for each additional compound to be tested.

h. Set compounds aside until they are ready to be added.

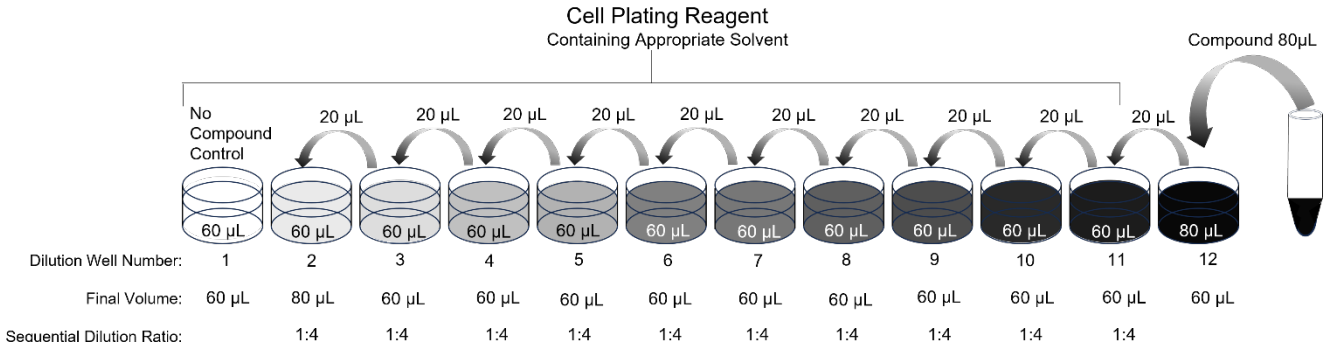
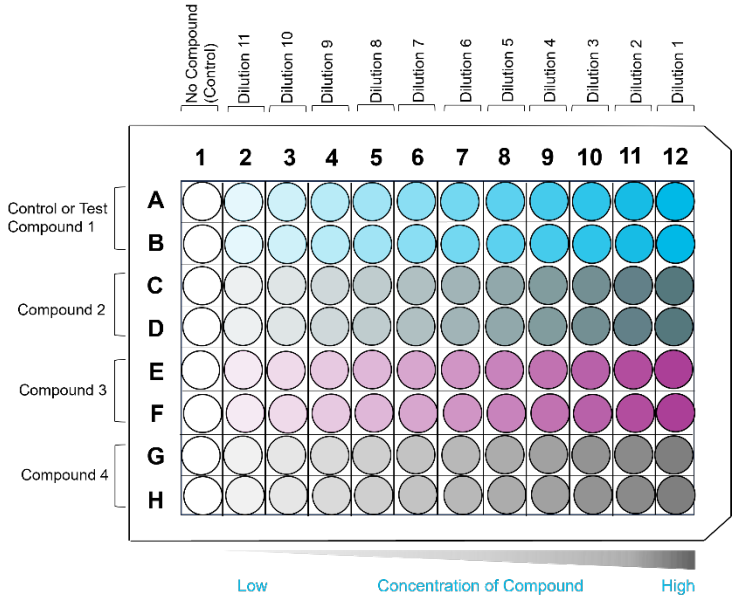


Figure 6. Compound Serial Dilutions

3. Remove the assay plate from the incubator and place it in the tissue culture hood.

   Transfer 10 µL of the compound dilution series from each row of the dilution plate to the corresponding wells of the assay plate, as shown in Figure 7.

   Incubate the assay plate at the specific temperature and time indicated on the target-specific datasheet.



The 96-well plate map shows an 11-point dose curve with 2 data points at each concentration, for each test compound with a 1:4 serial dilution scheme.

Figure 7. Representative Assay Plate Map

Section III: Detection Reagent Addition and Plate Reading

At this point, the compound treatment step has been completed. The following section contains procedures for adding the PathHunter® PL/PK Working Detection Solution and reading the assay plate on a luminometer.

Detection reagents must be prepared as a working solution prior to use.

Working Detection Solution		
Components	**Volume Ratio**	**Volume per Plate (mL)**
EA Reagent	1	2
Lysis Buffer	1	2
Substrate Reagent	4	8
Total Volume		**12**

Table 4. PathHunter® PL/PK Working Detection Solution Preparation

1. Prepare a stock of Working Detection Solution (Table 4) in a 15 mL polypropylene tube or reagent reservoir by mixing 1-part of EA Reagent, 1-part of Lysis Buffer and 4-part Substrate Reagent.

2. For a 96-well plate format, add 100 µL of Working Detection Solution (equivalent to 100% of assay volume) to all wells of the assay plate.

   Optional: Place the plate on an orbital shaker at 350 rpm for 1 minute to achieve uniform mixing.

3. Incubate the assay plate for 1 hour at room temperature in the dark.

   Working Detection Solution is light sensitive, thus incubation in the dark is necessary.

4. Read samples on a standard luminescence plate reader at 0.1 to 1 second/ well for photomultiplier tube readers or 5 to 10 seconds for imagers. The actual signal characteristics are affected over time by lab conditions such as temperature. The user should establish an optimal read time accordingly. Luminescence readers collect signal from all wavelengths. Some instrument manufacturers may include a cut-off filter at higher wavelengths, but usually no wavelength setting is needed for luminescence readout.

   The assay plate should be read within 2 hours after adding the detection reagent solution.

5. Data analysis can be performed using your choice of statistical analysis software (e.g., GraphPad Prism, Molecular Devices Softmax Pro, Biotek Instruments Gen5, Microsoft Excel, etc.).

Representative Data and Data Analysis

SPRINTer™ eXpress c-Myc Protein Turnover Biosensor Assay (HCT-116)
Cat. No. 91-1003E047CP0M

The following graph is an example of a typical dose-response curve using a c-Myc biosensor assay, for measuring MZ1-mediated turnover of the endogenous MYC protein. MZ1 promotes degradation of BRD4, a positive transcriptional regulator of c-Myc. Degradation of BRD4 leads to a reduction of MYC protein production. The data shows a potent, dose-dependent decrease in ED-MYC fusion protein levels in the SPRINTer™ eXpress c-Myc Protein Turnover Biosensor Assay (HCT-116), after an 18-hour incubation with MZ1.

The plate was read on the EnVision® Multimode Plate Reader and data analysis was conducted using GraphPad Prism 8.3.1.

Figure 8 shows a dose-response curve and Table 5 shows the EC_{50} and assay window observed for the decrease in MYC protein production when the cells are treated with MZ1.

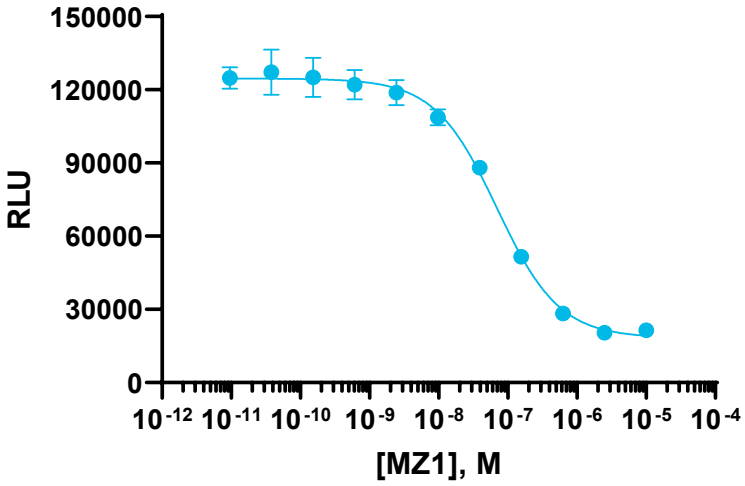


Figure 8. Dose Response Curve – MZ1-mediated turnover of endogenous MYC protein

EC_{50} (nM)	69.7
S/B	5.8

Table 5. EC_{50} and assay window – MZ1-mediated turnover of endogenous MYC protein

SPRINTer™ eXpress BTK Protein Turnover Biosensor Assay (K562)
Cat. No. 91-1005E042CP0M

The following graph 9 shows an example of a dose-response curve using a BTK biosensor assay for measuring DD 03-171 (TOCRIS, CA# 7160), a bi-functional degrader, mediated turnover of the endogenous BTK protein. The data shows a potent, dose-dependent decrease in BTK protein levels in the SPRINTer™ eXpress BTK Protein Turnover Biosensor Assay (K562) after an 18-hour incubation with DD 03-171.

The plate was read on the EnVision® Multimode Plate Reader and data analysis was conducted using GraphPad Prism 8.3.1.

Figure 9 shows a dose-response curve and Table 6 shows the EC50 and assay window observed for the decrease in BTK protein production when the cells are treated with a bi-functional degrader DD 03-171.

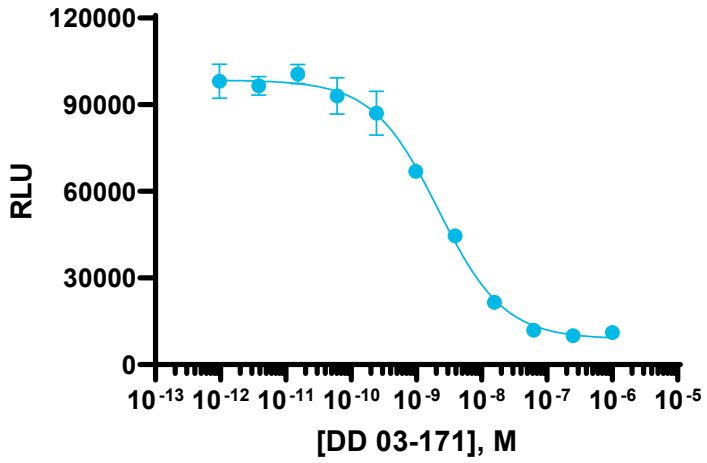


Figure 9. Dose Response Curve – DD-03-171-mediated turnover of endogenous BTK protein

EC_{50} (nM)	2.2
S/B	8.8

Table 6. EC_{50} and assay window – DD 03-171-mediated turnover of endogenous BTK protein

Supplemental Information

Running the Assay in a 384-well Plate

The eXpress kits are configured to run assays in a 96-well plate. The assay can be easily modified to run these in a 384-well plate by adjusting the volumes using the following guidelines (Table 7):

1. Suspend the cells in Cell Plating (CP) Reagent to a final volume of 4.8 mL.
2. Adjust volumes for the assay reagents for each protocol as indicated in the table below. The compound dilutions should be prepared at 5X the final screening concentration.

Assay Reagents	Volume per Well (µL)
Cell Suspension in CP Reagent	20
Compound Dilutions	5
Working Detection Solution	25
Total Assay Volume	**50**

Table 7. Assay Reagents for 384-well plate.

Frequently Asked Questions

How do you determine EC_{80} from the agonist reference curve?

- Determine the EC_{50} value and the Hill Slope from the agonist reference curve.
- Use an online EC_{80} calculator like QuickCalc by GraphPad (https://www.graphpad.com/quickcalcs/Ecanything1/), or
- Use the formula below where F is the percent response and H is the Hill Slope from the agonist reference curve:

$$EC_F = \left(\frac{F}{100 - F}\right)^{\frac{1}{H}} \times EC_{50}$$

- An example of EC_{80} calculation:

$$EC_F = \left(\frac{80}{100 - 80}\right)^{\frac{1}{H}} \times EC_{50}$$

What is the passage number of the frozen cells in the vials I receive when I purchase the cell line?

- The cells are shipped in passages 2 or 3, according to the cell line.

Can SPRINTer™ cell lines produce any viable viral particles?

- These cell lines cannot produce viable viral particles. SPRINTer™ cell lines are generated by CRISPR-mediated gene editing and no viral particle has been introduced into the original cell background.

What if there is no or low signal?

- Visually inspect the cells before and after compound incubation in a clear bottom plate to ensure that they are healthy. Make sure proper cell plating procedure is followed to ensure assay performance.
- Make sure that the cell line-specific detection reagents were used, stored and prepared as indicated in the datasheet.
- Make sure the proper assay mode is used (agonist mode or antagonist mode).
- Make sure the plate reader is setup properly to read luminescence.
- High levels of protein in the wells may interfere with enzyme fragment complementation. If high levels are present, a media exchange step could be performed just prior to the detection reagent addition. A mild detergent may also help decrease protein aggregation.
- White-walled assay plates should be used, since black-walled plates may decrease signal.

What if the response is lower than expected (lower than expected S:B)?

- Make sure that the ligand is prepared properly, take extra care to observe ligand solubility.
- Make sure DMSO and other solvents are not too high and not more than 1% final concentration.
- When testing ligands at high concentrations, make sure the vehicle is compatible.
- Make sure ligand is incubated for the designated time and at designated temperature.
- Make sure plates are protected from light during incubation.

What if the EC_{50} does not match reported values?

- Make sure ligands are incubated at the temperature indicated in the cell line-specific datasheet.
- The source of a ligand, particularly for biologics, can have a large effect on the observed pharmacology.
- Use fresh pipette tips during serial dilutions to avoid carryover.

What if the variability between replicates is high?

- Make sure dispensing equipment is properly calibrated and proper pipetting technique is used.
- Make sure to avoid cell clumps when plating. Uneven number of cells in the wells can cause high variability.

Do you perform any quantitative expression analysis of your recombinant cell lines?

- No. Because ED tag is introduced into the genomic locus of the target by CRISPR approach, the expression of the ED fusion protein reflects the endogenous level of the target at the given cell model. We select clones based on the best agonist response among similar expression levels.

Can I use my ligand, which is in a media containing high concentration of serum?

- Typically, our assays are highly tolerant to high serum content. However, there may be other ligands present in the serum that may raise the assay background, which can be target specific.
- We recommend that you aspirate the high serum media prior to adding detection reagents. Aspirating the media can help increase S/B, but it may not affect altered potency from ligands present in the serum or elevated background.

Do I need to use clear-bottom plates?

- We recommend using clear-bottom plates to visualize cells after plating. However, other plate formats can be used with some assay optimization.

Can these assays be run in 96-, 384-, and 1536-well formats?

- Yes. These assays can be used in high-throughput format.

What is the general liquid dispensing speed that you would recommend when using robotic dispensing instruments?

- This will depend on the particular instrument and has to be determined empirically, but in general, we would recommend a speed of 15 to 20 µL/second.

For additional information or Technical Support, see contact information at the bottom of this page.

Troubleshooting Guide

PROBLEM	CAUSE	SOLUTION
No response	Improper cell growth conditions	See datasheet for cell culture conditions
	High DMSO/solvent concentration	Maintain DMSO/solvent at <1% in serial dilutions of compounds
	Improper ligand used or improper ligand incubation time	See datasheet for recommended ligand and assay conditions
	Improper preparation of ligand (agonist or antagonist)	Refer to vendor specific datasheet to ensure proper handling, dilution, and storage of ligand
	Improper time course for induction	Optimize time course of induction with agonist and antagonist.
Decreased Response	Cells are not adherent and exhibit incorrect morphology	Confirm adherence of cells using microscopy
Low or No Signal	Improper preparation of detection reagents	Detection reagents should be prepared just prior to use and are sensitive to light.
	Problem with microplate reader	Microplate reader should be in luminescence mode. Read at 1 second/well.
Experimental S:B does not match datasheet value	For cell pools, S:B may vary greatly from passage to passage or day to day	Repeat the assay
		Confirm assay conditions
	Improper preparation of ligand (agonist or antagonist)	Some ligands are difficult to handle. Confirm the final concentration of ligands
EC_{50} is right-shifted	Improper ligand handling or storage	Check ligand handling requirements
	Difference in agonist binding affinity	Confirm that the ligand used is comparable to the ligand in the Product Insert
	Problems with plate type and compound stability	Hydrophobic compounds should be tested for solubility and may be diluted in buffer containing 0.1% BSA
		Non-binding surface plates may be necessary for hydrophobic compounds
High well-to-well variability in Z' study	Problems with plate type and compound solubility	Z' studies should be performed with automation
		It may be necessary to test plate types and compound stability

For additional information or technical support, please refer to the contact information at the bottom of this page.

Document Revision History

Revision Number	Date Released	Revision Details
0	March 2024	Initial version of SPRINTer™ eXpress Endogenous Protein Turnover Biosensor Assay User Manual

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