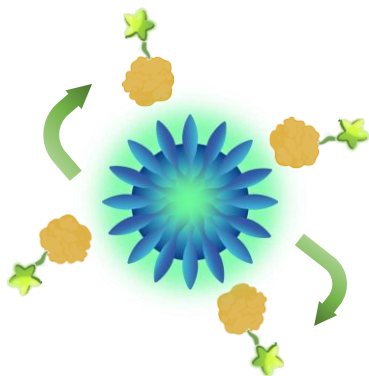


1 | PRESENTATION

ASSAY KIT DESCRIPTION



The NoW-Dtech™ Control Kit is designed to assess whether your reader is compatible with Bright-Dtech™ technology. It consists of donor nanoparticles and an acceptor-protein that binds to the nanoparticles and generates a TR-FRET signal.

The kit enables optimization of reader parameters to obtain a reliable standard curve, helping to maximize detection sensitivity and achieve an optimal signal-to-background ratio.

KIT COMPONENTS



Bright-Dtech™
Donor



Acceptor-Protein
(15 µM)



Control Kit
Buffer



384-well black
microplate

MATERIAL REQUIRED (NOT SUPPLIED)

- Vials.
- Precision single- and multichannel pipettes with disposable tips.
- One adhesive sealing film is included, but others will be necessary.
- Plate reader with TR-FRET option.

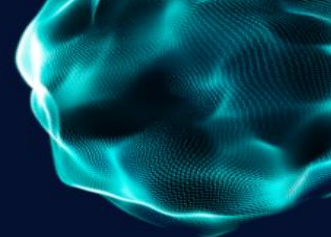
2 | REAGENTS AND SAMPLE PREPARATION

STORAGE AND PRECAUTIONS

- The kit must be stored at 2-8°C and used before the expiration date indicated on the box.
- Do not substitute or mix reagents from different lots or sources.
- We cannot guarantee the performance of the product if used outside the conditions described.
- Briefly spin down the tubes prior to use to remove any liquid that may have settled on the cap during shipping.
- Bring all reagents and plate, to room temperature prior to use, and homogenize them by vortexing. For the 'Bright-Dtech™ Donor' tube, sonication is strongly recommended instead of vortexing.



Important: Prepare the working standard dilutions, then distribute them into the wells. Next, prepare the donor solution and add it to the wells (see page 2).



PREPARATION OF WORKING STANDARD DILUTIONS

1. Spin down the '**Acceptor-Protein (15 µM)**' tube briefly before use.
2. Prepare the first standard point at 500 nM, using '**Control Kit Buffer**' as the diluent. Perform a 2-fold serial dilution to generate a 9-point standard curve, as shown in Table 1. This procedure will yield sufficient volume to run the standard dilution series in triplicate.
3. For Blank points, perform 6-9 replicates using '**Control Kit Buffer**' only.

Note: The diluted standards should be used immediately and must not be stored for future use.

Standard	Serial dilutions	Working concentration (nM)
Std 1	3.3 µL Acceptor-Protein (15 µM) + 96.7 µL Control Kit Buffer	500
Std 2	50 µL Std 1 + 50 µL Control Kit Buffer	250
Std 3	50 µL Std 2 + 50 µL Control Kit Buffer	125
Std 4	50 µL Std 3 + 50 µL Control Kit Buffer	62.5
Std 5	50 µL Std 4 + 50 µL Control Kit Buffer	31.3
Std 6	50 µL Std 5 + 50 µL Control Kit Buffer	15.6
Std 7	50 µL Std 6 + 50 µL Control Kit Buffer	7.81
Std 8	50 µL Std 7 + 50 µL Control Kit Buffer	3.91
Std 9	50 µL Std 8 + 50 µL Control Kit Buffer	1.95
Blank	100 µL Control Kit Buffer	0

Table 1: Preparation of serial dilutions for the standard curve

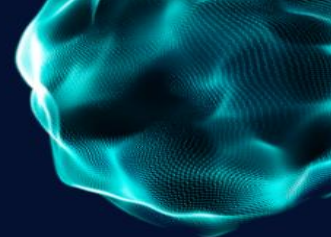
PREPARATION OF DONOR SOLUTION

The example below is for 36 points. For a different number of points, adjust the volumes.

 **Important:** Follow the order of addition exactly.

1. Sonicate (or vigorously vortex) the '**Bright-Dtech™ Donor**' tube to ensure a homogeneous solution.
2. Prepare a 1X solution of '**Bright-Dtech™ Donor**' using '**Control Kit Buffer**' as the diluent. Add 2 µL of '**Bright-Dtech™ Donor**' to a vial containing 398 µL of '**Control Kit Buffer**'.
3. Vortex thoroughly.

The donor solution is ready to be distributed into the wells.



3 | ASSAY PROTOCOL

PROTOCOL OVERVIEW

1. Add 7.5 µL of each working standard dilution.
2. Add 7.5 µL of the '**Bright-Dtech™ Donor 1X solution**' to each well.
3. Cover the plate with adhesive foil.
4. Incubate **1.5 h** at room temperature (RT).
5. Remove the adhesive foil.
6. Read the fluorescence at **520 nm** and at **535 nm** using a TR-FRET compatible microplate reader (excitation at **340 nm**).
7. Refer to '*TR-FRET plate reader settings*' section for detailed instructions.

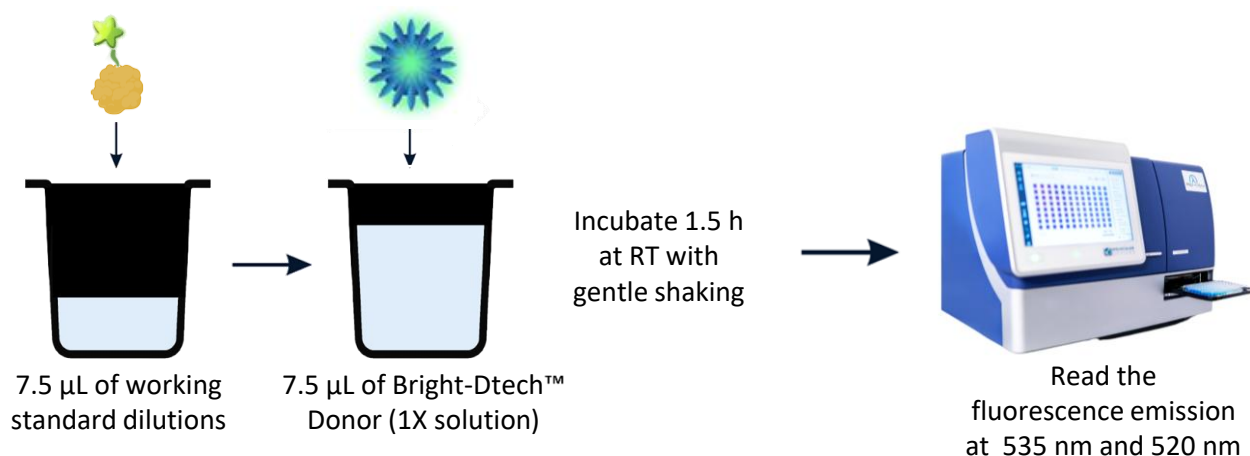


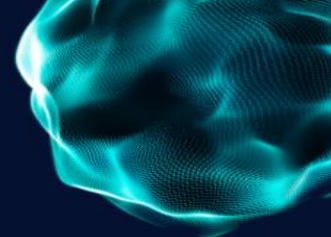
Figure 1: Assay procedure summary

TRF PLATE READER SETTINGS

- The instrument settings below are provided as a guideline only.
- Settings must be optimized for each reader (*).

Parameter	Setting
Excitation filter	340 nm
Emission filter	535 nm / 520 nm
Number of flashes *	110
Delay time *	30 µs
Integration time *	400 µs
Well scan	Multi-points

Table 2: Example of instrument settings for a TRF reader



5 | ANALYSIS

CALCULATIONS

- Calculate the TR-FRET Ratio for each well using the following formula:

$$\text{TR-FRET Ratio} = \frac{\text{Emission at 520 nm}}{\text{Emission at 535 nm}} \times 10^4$$

- Calculate the coefficient of variation (CV%) for each triplicate to assess the precision and consistency of the assay results using the following formula. The smaller the percentage, the more precise and consistent the data.

$$\text{CV\%} = \frac{\text{Standard deviation of the triplicate}}{\text{TR-FRET mean ratio of the triplicate}} \times 100$$

- Create a standard curve by plotting the TR-FRET Ratio for each set of triplicate standards on the Y-axis against the corresponding Acceptor-Protein concentration on the X-axis.
- The best-fit standard curve can be determined using nonlinear regression with a four- or five-parameter logistic (4PL or 5PL) curve fit (sigmoidal dose-response curve with variable slope). We recommend using a commercial software program for this analysis.
- The intensity of the resulting signal is directly proportional to the concentration of Acceptor-Protein.

6 | PERFORMANCE CHARACTERISTICS

VALIDATION

The purpose of this kit is to determine the optimal reader parameters to achieve the following performance

criteria:

- LOQ below 1 nM

- EC50 close to 20 nM

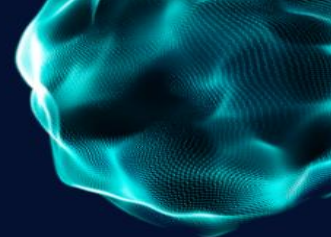
- Signal-to-noise ratio greater than 10 at 62.5 nM Acceptor-Protein, calculates as:

$$\text{Signal-to-noise Ratio} = \frac{\text{TR-FRET Ratio Std 62.5 nm}}{\text{TR-FRET Ratio Blank}}$$

Note: If these performance criteria cannot be achieved, Now-Dtech™ kits can still be used, but may operate with reduced performance.

Format	384-well plate
Hands-on time	20-30 minutes
Incubation time	1.5 h
Limit of Detection (LOD)	0.650 nM
EC ₅₀	20 nM
Dynamic range	1 – 500 nM

Table 3: Validation parameters resume



TYPICAL STANDARD CURVE

The standard curve below is an example generated using the SpectraMax ID5 reader from Molecular Devices. Results may vary between different TRF readers.

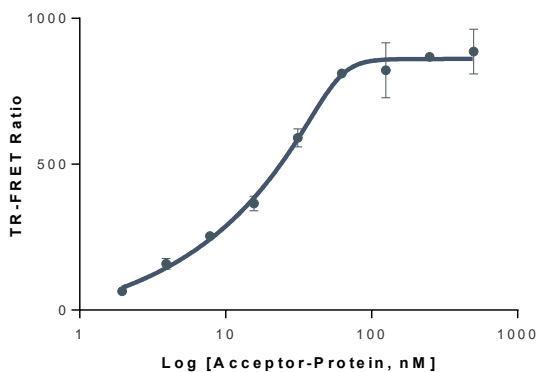


Figure 2: A representative standard curve

7 | TROUBLESHOOTING GUIDE

Problem	Possible cause	Solution
Low or poor standard curve	Inaccurate pipetting.	Check pipettes.
	Improper standard dilution.	Before opening, briefly spin the standard stock tube. Check calculations and create a new standard curve.
	Incorrect procedure.	Check the protocol (ensure reagents were added in the correct order).
No or weak signal	Incubation times too short.	Ensure sufficient incubation times; switch to a 2.5 hours to overnight standard/sample incubation.
	Inadequate reagent volumes or improper dilution.	Check pipettes and ensure correct preparation (review protocol).
	Incorrect procedure.	Check protocol (ensure reagents were added in the correct order).
	Incorrect reader settings.	Check filters/reader settings (ensure the plate reader is set accurately for the type of detection being used).
High CV%	Bright-Dtech™ Donor tube not mixed properly.	Sonicate or mix vigorously the Bright-Dtech™ Donor tube.
Low sensitivity	Improper storage of the kit.	All reagents should be stored according to the instructions.