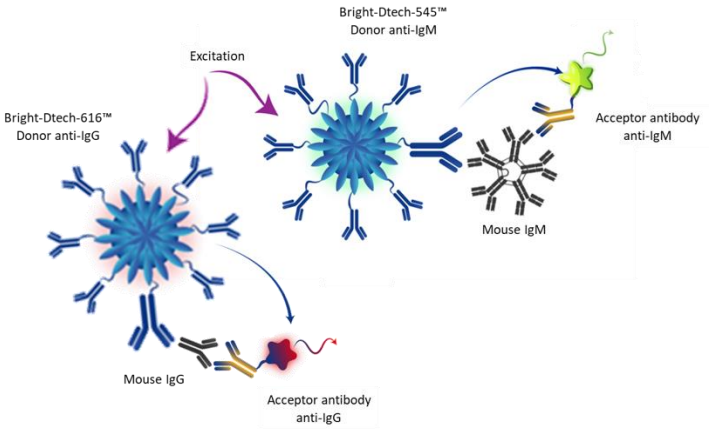
POLY-DTECH
Enhance Your Detection

Multi-Dtech™
Mouse IgG/IgM Assay Kit
Ref: FRETmIgG/IgM

1 | PRESENTATION

ASSAY KIT DESCRIPTION

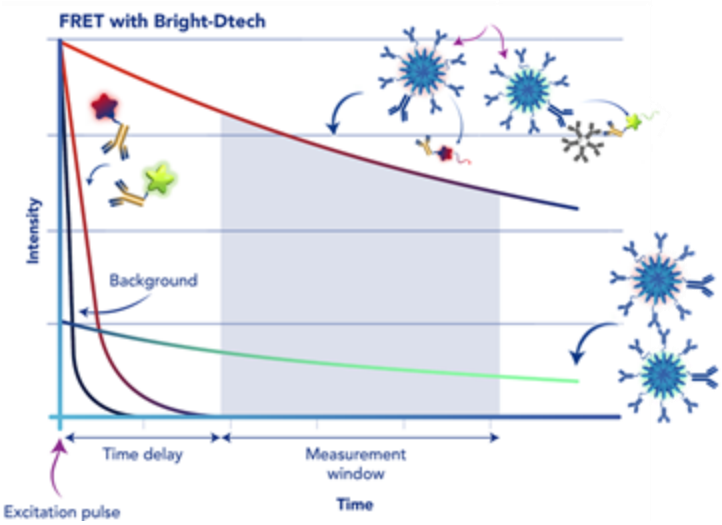


The Mouse IgG/IgM assay kit enables the simultaneous quantitative detection of mouse IgG and IgM in serum using the TR-FRET methodology. The kit incorporates Bright-Dtech technology, which enhances detection through exceptional specificity and sensitivity. The assay range is 1 to 1000 ng/ml

TR-FRET ASSAY PRINCIPLE

Time-Resolved Förster Resonance Energy Transfer (TR-FRET) is a cutting-edge immunoassay technique widely used for the detection and quantification of biomolecules, including Mouse IgG and IgM in serum. This method is fast, wash-free, reduces background fluorescence, and is cost-effective. Our Bright-Dtech™ technology offers a more sensitive (high brightness, leading to improved limits of detection) and stable (high photo-stability over days or weeks, resistance to photo-bleaching, and sustained signal intensity) approach.

In the Multi-Dtech™ TR-FRET method, two pairs of antibodies are employed to specifically detect mouse IgM and IgG. The donor antibodies are conjugated to our fluorescent nanoparticles, while the acceptor antibodies are labeled with fluorescent molecules. When the targets are recognized by the antibody pairs, energy is transferred from the donor to the acceptor, generating two distinct signals for each target. These signals are simultaneously measured using a time-resolved fluorescence reader, yielding highly specific and quantitative results.



2 | MATERIALS

KIT COMPONENTS

Mouse IgM/IgG (Standard stock 10X)

Bright-Dtech-616™ anti-IgG Donor

Bright-Dtech-545™ anti-IgM Donor

Acceptor antibodies

TRF Buffer

384-well black microplate

MATERIALS REQUIRED BUT 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.

3 | 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, plate, standard, and samples to room temperature prior to use, and homogenize them by vortexing. For the **'Bright-Dtech™ Donor antibody'** tube, sonication is strongly recommended instead of vortexing.

Important: Prepare the working standard dilutions and the samples first (see pages 3 and 4), then distribute them into the wells. Next, prepare the donor/acceptor mix solution (see page 4) and add it to the wells.

PREPARATION OF WORKING STANDARD DILUTIONS

1. Spin down the **'Standard stock (10X)'** tube briefly before use.
2. Prepare a 1X solution of **'Standard stock'** using **'TRF Buffer'** as the diluent. Add 1 µL of **'Standard stock (10X)'** to a vial containing 9 µL of **'TRF Buffer'**.
3. Use this 1X solution to prepare the first standard point at 1 000 ng/mL, using **'TRF Buffer'** as the diluent. Perform a 2-fold serial dilution to generate an 11-point standard curve, as shown in Figure 1. This procedure will yield sufficient volume to run the standard dilution series in duplicate.
4. For Blank points, perform 10 replicates using **'TRF Buffer'** alone.

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

Standard	Serial dilutions	Working concentration (ng/mL)
Std 1X	1 µL Std stock (10X) + 9 µL TRF Buffer	-
Std 1	2 µL Std 1X + 98 µL TRF Buffer	1 000
Std 2	40 µL Std 1 + 40 µL TRF Buffer	500
Std 3	40 µL Std 2 + 40 µL TRF Buffer	250
Std 4	40 µL Std 3 + 40 µL TRF Buffer	125
Std 5	40 µL Std 4 + 40 µL TRF Buffer	62.5
Std 6	40 µL Std 5 + 40 µL TRF Buffer	31.25
Std 7	40 µL Std 6 + 40 µL TRF Buffer	15.6
Std 8	40 µL Std 7 + 40 µL TRF Buffer	7.8
Std 9	40 µL Std 8 + 40 µL TRF Buffer	3.9
Std 10	40 µL Std 9 + 40 µL TRF Buffer	1.9
Std 11	40 µL Std 10 + 40 µL TRF Buffer	0.98
Blank	150 µL TRF Buffer	0

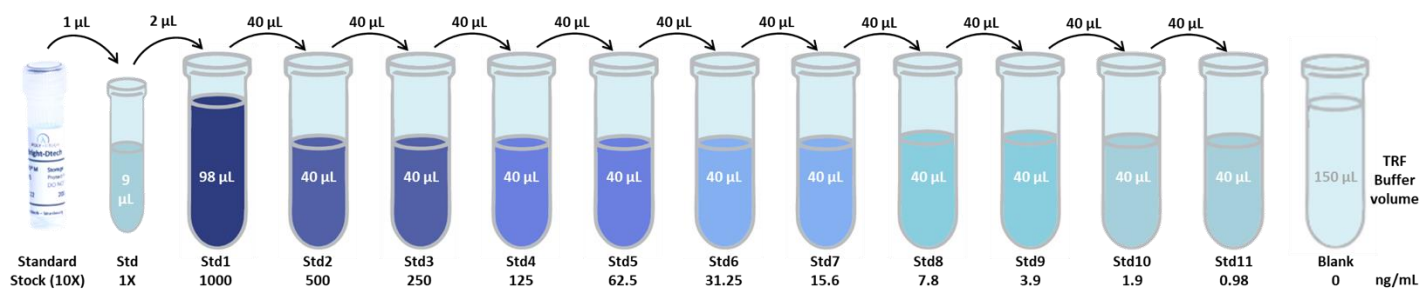


Figure 1: *Preparation of serial dilutions for the standard curve*

SAMPLE PREPARATION

- For serum sample dilutions: use TRF Buffer.
- Suggested dilution for normal serum : 10 000-fold or more.
- Prepare enough volume to add 10 µL per well (e.g., 40 µL if using duplicates).
- Take care to gently agitate patient samples to ensure homogeneity.

Please note that levels of the target protein may vary between different samples. The optimal dilution factor for each sample must be determined by the investigator.

PREPARATION OF DONOR/ACCEPTOR MIX SOLUTION

The example below is for 384 points (an entire plate). For a different number of points, adjust the volumes as indicated in Table 1.

Important: *Follow the order of addition exactly.*

1. Sonicate (or vigorously vortex) the coupled nanoparticles **'Bright-Dtech™ Donor antibody'** to ensure a homogeneous solution.
2. Add 36 µL of **'Bright-Dtech-545™ anti-IgM Donor'** stock solution to a vial containing 2 311 µL of **'TRF Buffer'** and vortex.
3. Add 36 µL of **'Bright-Dtech-616™ anti-IgG Donor'** and vortex.
4. Allow to stand for 5 minutes before adding the acceptor antibody.
5. Add 17.5 µL of **'Acceptor antibodies'** to this solution and vortex again

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

Note: *The 1X Acceptor antibody solution should be used immediately and must not be stored for future use.*

Points	96	192	288	384
Bright-Dtech-545™ anti-IgM Donor	9 µL	18 µL	27 µL	36 µL
Bright-Dtech-616™ anti-IgG Donor	9 µL	18 µL	27 µL	36 µL
TRF Buffer	578 µL	1155 µL	1733 µL	2310 µL
Acceptor antibody	4.4 µL	8.8 µL	13.1 µL	17.5 µL

Table 1: *Preparation of Donor/Acceptor mix solution*

4 | ASSAY PROTOCOL

PROTOCOL OVERVIEW

1. Add 10 µL of each working standard or 10 µL of diluted sample to the appropriate wells.
2. Add 5 µL of the Donor/Acceptor mix solution to each well.
3. Cover the plate with adhesive foil.
4. Incubate for **1h30** at room temperature (RT).
5. Remove the adhesive foil.
6. Read the fluorescence at **520, 535, 616 and 665 nm** using a TR-FRET-compatible microplate reader (excitation at **340 nm**).
7. Refer to '*TR-FRET plate reader settings*' section for detailed instructions.

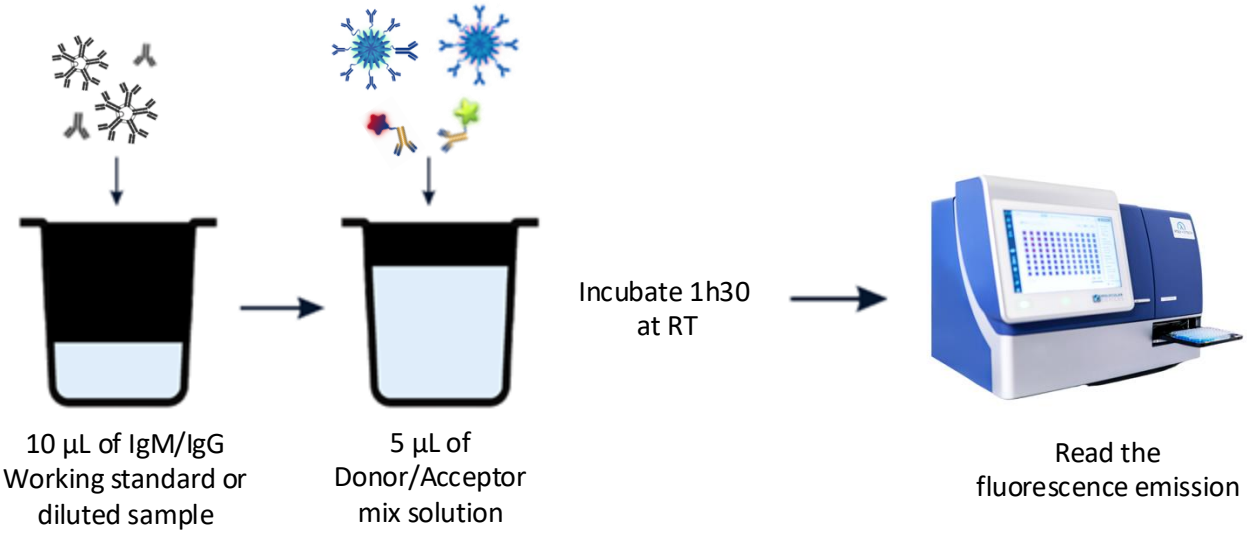


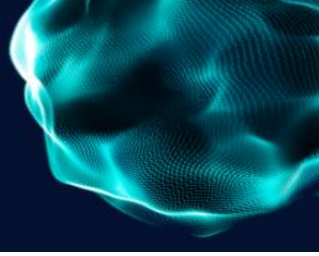
Figure 2: *Assay procedure summary*

TRF PLATE READER SETTINGS

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

Parameter	Setting
Excitation filter	340 nm
Emission filter for IgM detection	520 nm / 535 nm
Emission filter for IgG detection	616 nm / 665 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 :

IgM	IgG
$\text{Ratio NEG-IgM} = \frac{\textbf{Emission at 520 nm (Std Blank)}}{\textbf{Emission at 535 nm (Std Blank)}} \times 104$	$\text{Ratio NEG-IgG} = \frac{\textbf{Emission at 616 nm (Std Blank)}}{\textbf{Emission at 665 nm (Std Blank)}} \times 104$
$\text{TR-FRET Ratio-IgM} = \frac{\textbf{Emission at 520 nm}}{\textbf{Emission at 535 nm}} \times 10^4 - \textit{Ratio NEG-IgM}$	$\text{TR-FRET Ratio-IgG} = \frac{\textbf{Emission at 616 nm}}{\textbf{Emission at 665 nm}} \times 10^4 - \textit{Ratio NEG-IgG}$

– Create two standard curves (one for IgG and one for IgM) by plotting the TR-FRET ratio for each set of standards in duplicate or triplicate on the Y axis against the corresponding concentration of mouse IgM and IgG 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.

– To determine the unknown Mouse IgG and IgM concentrations in samples, locate the TR-FRET Ratio values, insert them into the standard curve equation, and calculate the expected concentration. If the samples have been diluted, the concentration obtained from the standard curve must be multiplied by the dilution factor.

– The intensity of the resulting signal is directly proportional to the concentration of the antigen present in the sample.

– Any undiluted sample reading greater than the highest standard should be diluted appropriately with ‘TRF Buffer’ and retested.

– Any diluted sample reading greater than the highest standard should be further diluted appropriately with ‘TRF Buffer’ and retested.

– Any diluted sample reading lower than the lowest standard should be less diluted appropriately with ‘TRF Buffer’ and retested.

– A high dose hook effect may occur in samples with very high Mouse IgG and IgM concentrations. In this case, dilute the samples further to avoid this effect.

TYPICAL STANDARD CURVE

The standard curve below is an example only and should not be used to calculate results for unknown samples.

Results may vary between different TRF readers.

A new standard curve must be generated with each assay.

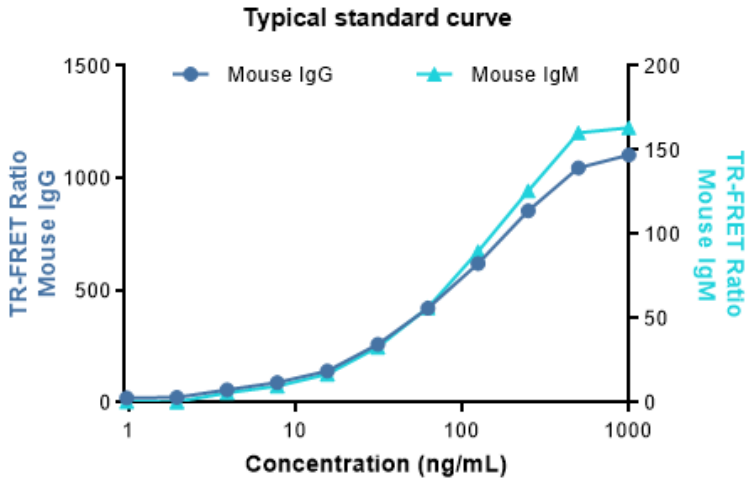


Figure 3: *A representative standard curve*

6 | PERFORMANCE CHARACTERISTICS

VALIDATION

Assay Type	Homogeneous sandwich immunoassay with Bright-Dtech™
Format	384-well plate
Hands-on time	20-30 minutes
Incubation time	1h30
Sample type (volume)	Diluted serum (10 µL)
Specificity	Mouse IgM and IgG
Limit of Detection (LOD)	IgM : 2 ng/mL IgG : 0.72 ng/mL
EC_{50}	IgM : 110 ng/mL IgG : 110 ng/mL
Dynamic range	IgM : 2 - 1000 ng/mL IgG : 1 - 1000 ng/mL

Table 3: *Validation parameters resume*

SENSITIVITY

The Limit of Detection (LOD) for IgM is **4 ng/mL and** IgG is **0.72 ng/mL** This was determined by adding three standard deviations to the mean value of 100 blank (zero) standard replicates, run across three independent assays, and calculating the corresponding concentration.

PRECISION

Intra-assay precision

Intra-assay precision was evaluated by measuring three concentrations (high, medium, and low) of Mouse IgG and IgM spiked in TRF Buffer, with 20 replicate determinations. The intra-assay percentage coefficient of variation (CV%) was < 10%.

Spike	Number of replicates	%CV	
		IgM	IgG
High	20	8	9
Medium	20	6	7
Low	20	9	9

Table 4: *Intra-assay precision performance*

Inter-assay precision

Inter-assay precision was evaluated by measuring three concentrations (high, medium, and low) of Mouse IgG and IgM spiked in TRF Buffer, with 20 replicates determinations in three independent assays. The inter-assay percentage coefficient of variation (CV%) was < 12%.

Spike	Number of replicates	%CV	
		IgM	IgG
High	3 x 20	11	9
Medium	3 x 20	4	2
Low	3 x 20	5	2

Table 5: *Inter-assay precision performance*

RECOVERY

To determine whether Mouse IgG and IgM detection is affected by differences in the standard curve diluent and biological sample matrix, three different concentrations (high, medium, and low) of Mouse IgG and IgM were spiked into Mouse serum depleted of IgG and IgM, diluted 1/10000.

Spike	% Recovery	
	IgM	IgG
High	101	91
Medium	100	110
Low	97	109

Table 6: *Recovery performance*

LINEARITY

To evaluate the linearity of the assay, a high concentration of Mouse IgG and IgM was spiked into Mouse serum depleted of IgG and IgM, diluted 1/50 000. The sample was then serially diluted 2-fold up to 1/64 using TRF Buffer.

Dilution factor	% Linearity	
	IgM	IgG
1/2	94	120
1/4	103	107
1/8	100	104
1/16	98	94
1/32	107	94
1/64	90	96
1/128	99	103

Table 7: *Linearity performance*

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 antibody not mixed properly.	Sonicate or mix vigorously the Bright-Dtech™ Donor antibody tube.
Low sensitivity	Improper storage of the kit.	All reagents should be stored according to the instructions.