MANUAL

HUMAN GRANZYME B KITS

Part # 62HGRZBPEG & 62HGRZBPEH

Test Size#: 500 tests (62HGRZBPEG) and 10,000 tests (62HGRZBPEH)

Assay volume: 20µL

Revision: #06 of September 2023

Store at: -16°C or below (62HGRZBPEG); -16°C or below (62HGRZBPEH)

For research use only. Not for use in diagnostic procedures.

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of human Granzyme B in cell/tissue culture supernatants and whole cells and offers a fast alternative to ELISA. The assay is compatible with mouse Granzyme B.

The detection principle of this kit is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1, Granzyme B is detected in a sandwich assay by using anti Granzyme B antibody labeled with Europium cryptate (donor), and anti-Granzyme B antibody labeled with d2 (acceptor).

When the dyes are in close proximity, the excitation of the donor with a light source (laser or flash lamp) triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665 nm). Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the Granzyme B concentration.

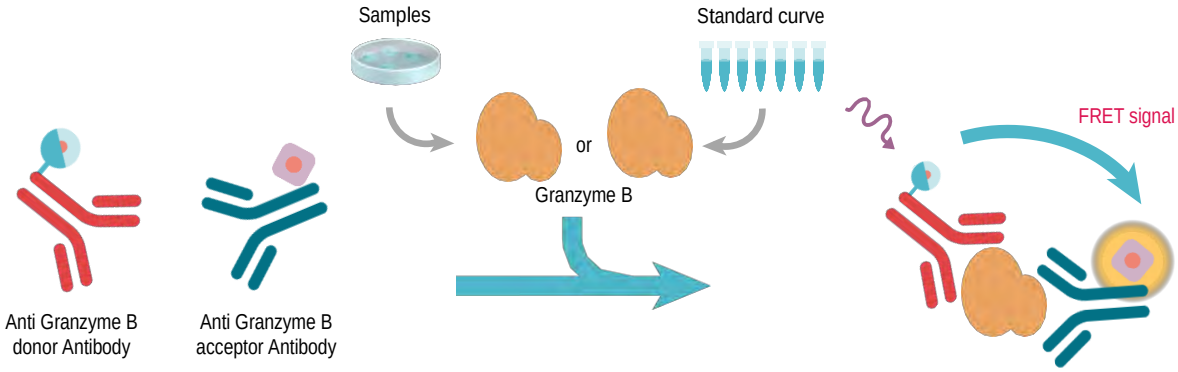
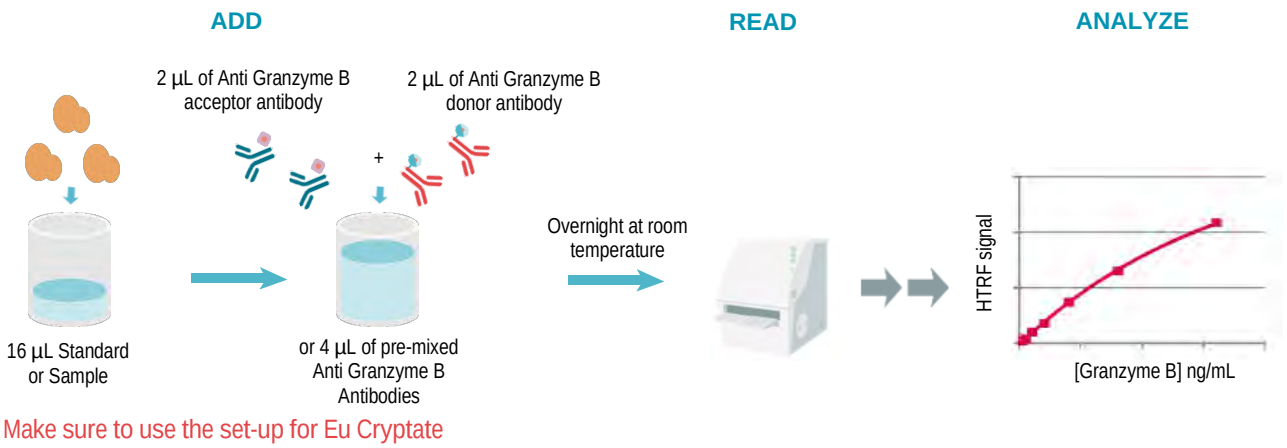


Figure 1. Principle of HTRF Granzyme B sandwich assay

MANUAL AT A GLANCE



Make sure to use the set-up for Eu Cryptate

MATERIALS

KIT COMPONENTS	500 TESTS*	10,000 TESTS*
Granzyme B Standard Lyophilized	1 vial 4500 ng/mL	1 vial 4500 ng/mL
Granzyme B Eu Cryptate Antibody Frozen 50X	1 vial 20 µL	1 vial 0.4 mL
Granzyme B d2 Antibody Frozen 50X	1 vial 20 µL	1 vial 0.4 mL
Diluent** #5 5X	1 vial 2 mL	1 vial 10 mL
Detection Buffer*** #3 Ready-to-use	2 vials 1.5 mL	1 vial 50 mL

* When used as advised, the two available kit sizes will provide sufficient reagents for 500 tests and 10,000 tests respectively in 20 µL final volume. Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.
** Medium like cell culture medium can be an alternative to the diluent.
*** The Detection buffer is used to prepare working solutions of acceptor and donor reagents.

Purchase separately

- HTRF®-Certified Reader. Make sure the setup for Eu Cryptate is used. For a list of HTRF-compatible readers and set-up recommendations, please visit www.revvity.com
- Small volume (SV) detection microplates. For more information about microplate recommendations, please visit our website at: www.revvity.com

STORAGE AND STABILITY

KIT:

- Store the kit at -16°C or below.
- Under proper storage conditions, reagents are stable until the expiry date indicated on the label.
- Diluent and detection buffer are shipped frozen, but can be stored at 2-8°C in your premises.

REAGENTS:

- If lyophilized, reconstituted reagents, antibodies, and standard stock solutions may be frozen and thawed only once. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.
- Volume of Human Granzyme B standard aliquots should not be under 10 µL.
- Thawed diluent and detection buffer can be stored at 2-8°C in your premises.

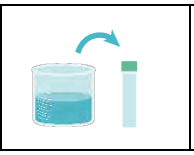
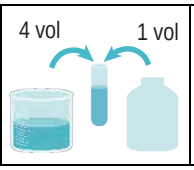
REAGENT PREPARATION

BEFORE YOU BEGIN

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Thaw the frozen reagents at room temperature, allow them to warm up to room temperature for at least 30 mins before use
- Before use, allow Diluent and Detection buffer to warm up at room temperature and homogenize them with a vortex.
- It is recommended to filter buffers.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- Granzyme B standards (for standard curve) must be prepared in diluent or in the same medium as the samples.

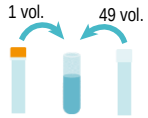
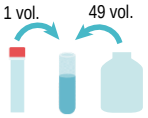
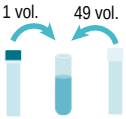
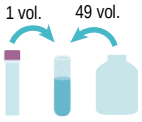
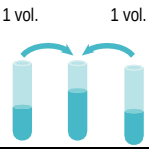
TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS		10,000 TESTS
Anti-Granzyme B Eu Cryptate antibody		
Thaw the Granzyme B Eu Cryptate antibody. Mix gently. This 50X stock solution can be frozen and stored at -60°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.		Thaw the Granzyme B Eu Cryptate antibody. Mix gently. This 50X stock solution can be frozen and stored at -60°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.
Anti- Granzyme B d2 antibody		
Thaw the Granzyme B d2 antibody. Mix gently. This 50X stock solution can be frozen and stored at -60°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.		Thaw the Granzyme B d2 antibody. Mix gently. This 50X stock solution can be frozen and stored at -60°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below..
Granzyme B Standard		
Thaw the Granzyme B standard stock solution (200 ng/ mL) at RT. Mix gently. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solution into disposable plastic vials for storage at -60°C or below.		Thaw the Granzyme B standard stock solution (200 ng/ mL) at RT. Mix gently. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solution into disposable plastic vials for storage at -60°C or below.
Diluent		
Dilute 5-fold the 5 X diluent #5 with distilled water: homogenize the 5 X diluent #5 with a vortex and add 1 volume of stock solution in 4 volumes of distilled water (e.g., 1 mL of diluent + 4 mL of distilled water). Mix gently after dilution. This 1X diluent can be frozen and stored at -60°C or below.		Dilute 5-fold the 5 X diluent #5 with distilled water: homogenize the 5 X diluent #5 with a vortex and add 1 volume of stock solution in 4 volumes of distilled water (e.g., 10 mL of diluent + 40 mL of distilled water). Mix gently after dilution. This 1X diluent can be frozen and stored at -60°C or below.
Detection buffer		
The Detection buffer is ready-to-use.		

TO PREPARE WORKING ANTIBODY SOLUTIONS:

Each well requires 2 µL of Granzyme B-Eu Cryptate Antibody and 2 µL of Granzyme B-d2 Antibody.
Prepare the two antibody solutions in separate vials

500 TESTS		10,000 TESTS	
Granzyme B Eu Cryptate antibody			
Dilute 50-fold the 50X stock solution (thawed reagent) of Granzyme B Eu Cryptate antibody with Detection buffer #3: add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of detection buffer (e.g. 20 µL of Eu Cryptate antibody stock solution + 980 µL of detection buffer).			Dilute 50-fold the 50X stock solution (thawed reagent) of Granzyme B Eu Cryptate antibody with Detection buffer #3: add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of detection buffer (e.g. 0.4 mL of Eu Cryptate antibody stock solution + 19.6 mL of detection buffer).
Granzyme B d2 antibody			
Dilute 50-fold the 50X stock solution (thawed reagent) of Granzyme B d2 antibody with Detection buffer #3: add 1 volume of d2 antibody stock solution in 49 volumes of detection buffer (e.g. 20 µL of d2- antibody stock solution + 980 µL of detection buffer).			Dilute 50-fold the 50X stock solution (thawed reagent) of Granzyme B d2 antibody with Detection buffer #3: add 1 volume of d2 antibody stock solution in 49 volumes of detection buffer (e.g. 0.4 mL of d2 antibody stock solution + 19.6 mL of detection buffer).
Antibody mix			
It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 1 mL of d2 antibody + 1 mL of Cryptate antibody).			It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 20 mL of d2 antibody + 20 mL of Cryptate antibody).

TO PREPARE WORKING STANDARDS SOLUTIONS:

- Each well requires 16 µL of standard.
- Dilute the standard stock solution serially with diluent #5 (1X)
- If culture medium is used to dilute the standard, we recommend to supplement it with serum (2 to 10%) or BSA (0.2 to 1%) in order to avoid Granzyme B sticking to assay plates.
- In order to check for a potential interference effect from your own assay buffer when using the assay for the first time, we highly recommend the parallel preparation of a standard curve in your own supplemented cell culture medium and in diluent #5 (1X).
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

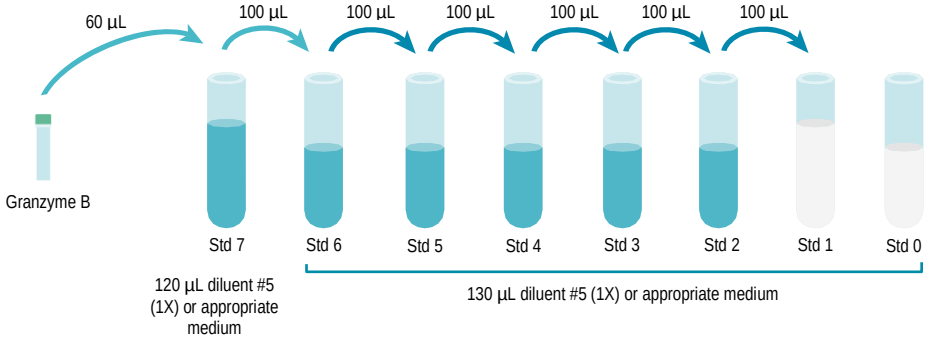
A recommended standard dilution procedure is listed and illustrated below:

Dilute the standard stock solution 3-fold with diluent #5 (1X) to prepare high standard (Std 7): e.g. take 60 µL of standard stock solution and add it to 120 µL of diluent #5 (1X). Mix gently.

Use the high standard (Std 7) to prepare the standard curve using 1/2.3 serial dilutions as follows:

- Dispense 130 µL of diluent #5 (1X) in each vial from Std 6 to Std 0.
- Add 100 µL of standard to 140 µL of diluent #5 (1X), mix gently and repeat the 1/2.4 serial dilution to make standard solutions: std6, std5, std4, std3, std2, std1.

This will create 7 standards for the analyte. Std 0 (Negative control) is diluent #5 (1X) or appropriate culture medium alone



STANDARD	SERIAL DILUTIONS	WORKING SOLUTIONS
Standard Stock solution	Reconstituted lyophilisate	4 500 ng/mL
Standard 7	60 µL Standard stock Solution + 120 µL diluent 5 (1X)	1 500 ng/mL
Standard 6	100 µL standard 7 + 130 µL diluent 5 (1X)	652 ng/mL
Standard 5	100 µL standard 6 + 130 µL diluent 5 (1X)	283 ng/mL
Standard 4	100 µL standard 5 + 130 µL diluent 5 (1X)	123 ng/mL
Standard 3	100 µL standard 4 + 130 µL diluent 5 (1X)	54 ng/mL
Standard 2	100 µL standard 3 + 130 µL diluent 5 (1X)	23 ng/mL
Standard 1	100 µL standard 2 + 130 µL diluent 5 (1X)	10 ng/mL
Standard 0	130 µL diluent 5 (1X)	0

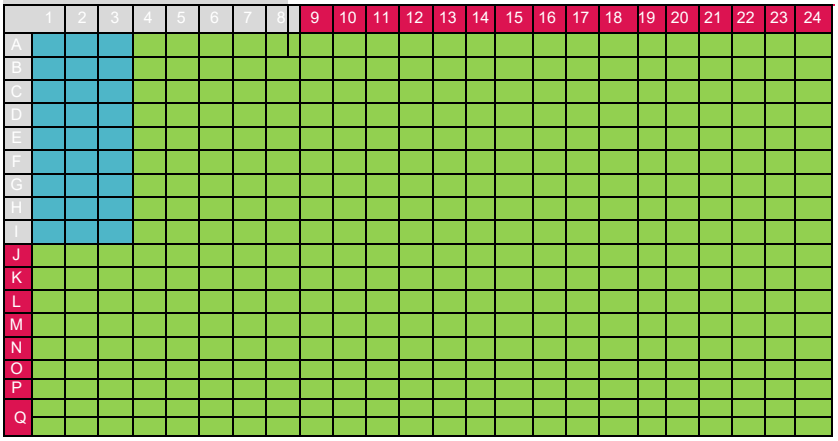
TO PREPARE SAMPLES

- Each well requires 16 µL of sample.
- Just after their collection, put the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at -60°C or below. Avoid multiple freeze/thaw cycles.
- Cell supernatants must be prepared using a culture medium supplemented with serum (2 to 10%) or BSA (1%) to avoid Granzyme B sticking to culture vessels.
- Samples with a concentration above the highest standard (Std 7) must be diluted diluent #5 (1X)

ASSAY MANUAL

	STANDARD (STD 0 - STD 7)	SAMPLES
tep 1	Dispense 16 µL of each Granzyme B standard (Std 0 - Std 7) into each standard well	Dispense 16 µL of each sample into each sample well.
Step 2	Add 2 µL of Granzyme B d2 antibody working solution to all wells	
Step 3	Add 2 µL of Granzyme B Eu Cryptate antibody working solution to all wells.	
Step 4	Seal the plate and incubate overnight at RT	
Step 5	Remove the plate sealer and read on an HTRF® compatible reader	

	1	2	3	4	5	6
A	16 µL Std 0 (Negative control) 2 µL Granzyme B-d2 2 µL Granzyme B -Eu Cryptate	Repeat Well A1	Repeat Well A1	16 µL sample 1 2 µL Granzyme B-d2 2 µL Granzyme B-Eu Cryptate	Repeat Well A4	Repeat Well A4
B	16 µL Std 1 2 µL Granzyme B -d2 2 µL Granzyme B -Eu Cryptate	Repeat Well B1	Repeat Well B1	16 µL sample 2 2 µL Granzyme B-d2 2 µL Granzyme B-Eu Cryptate	Repeat Well B4	Repeat Well B4
C	16 µL Std 2 2 µL Granzyme B-d2 2 µL Granzyme B -Eu Cryptate	Repeat Well C1	Repeat Well C1	16 µL sample 3 2 µL Granzyme B-d2 2 µL Granzyme B-Eu Cryptate	Repeat Well C4	Repeat Well C4
D	16 µL Std 3 2 µL Granzyme B -d2 2 µL Granzyme B -Eu Cryptate	Repeat Well D1	Repeat Well D1	16 µL sample … 2 µL Granzyme B-d2 2 µL Granzyme B-Eu Cryptate	Repeat Well D4	Repeat Well D4
E	16 µL Std 4 2 µL Granzyme B -d2 2 µL Granzyme B -Eu Cryptate	Repeat Well E1	Repeat Well E1	16 µL sample … 2 µL Granzyme B-d2 2 µL Granzyme B-Eu Cryptate	Repeat Well E4	Repeat Well E4
F	16 µL Std 5 2 µL Granzyme B -d2 2 µL Granzyme B -Eu Cryptate	Repeat Well F1	Repeat Well F1	16 µL sample … 2 µL Granzyme B-d2 2 µL Granzyme B-Eu Cryptate	Repeat Well F4	Repeat Well F4
G	16 µL Std 6 2 µL Granzyme B -d2 2 µL Granzyme B -Eu Cryptate	Repeat Well G1	Repeat Well G1	16 µL sample … 2 µL Granzyme B-d2 2 µL Granzyme B-Eu Cryptate	Repeat Well G4	Repeat Well G4
H	16 µL Std 7 2 µL Granzyme B -d2 2 µL Granzyme B -Eu Cryptate	Repeat Well H1	Repeat Well H1	16 µL sample … 2 µL Granzyme B-d2 2 µL Granzyme B-Eu Cryptate	Repeat Well H4	Repeat Well H4



DATA REDUCTION & INTERPRETATION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV}(\%) = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

3. Calculate the delta ratio of the acceptor and donor emission signals for each individual well. The Standard 0 (Negative control) plays the role of an internal assay control.

delta Ratio = Ratio Standard or sample - Ratio Standard 0

For more information about data reduction, please visit www.revvity.com

RESULTS

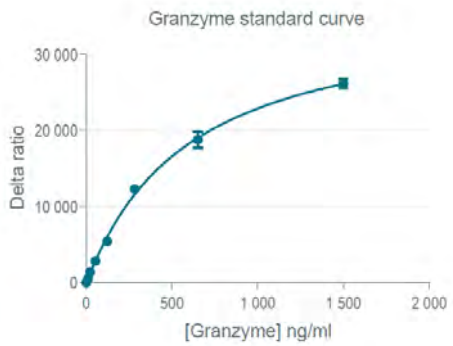
This data must not be substituted for the data obtained in the laboratory, and should be considered only as an example.

Results may vary from one HTRF® compatible reader to another.

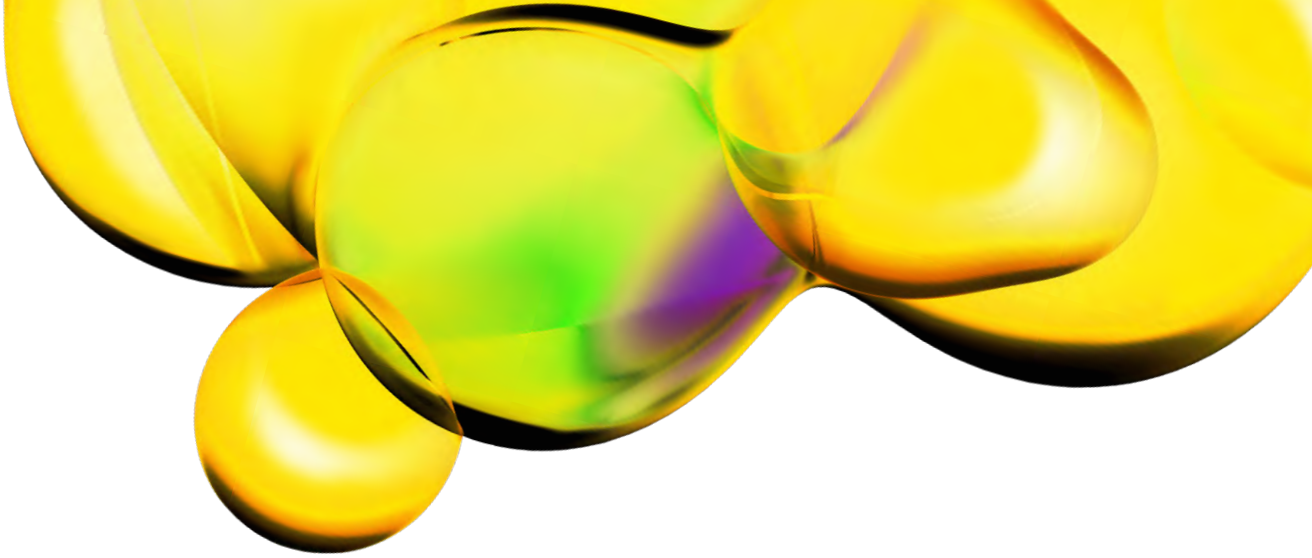
Standard curve fitting with the 4 Parameter Logistic (4PL with 1/Y²) model

For more information about curve fitting please visit www.revvity.com

		Ratio (1)	CV% (2)	Delta Ratio (3)
Standard 0	Negative control	1158	2%	0
Standard 1	10 ng/mL	1658	3%	500
Standard 2	23 ng/mL	2537	4%	1 379
Standard 3	54 ng/mL	4004	9%	2 846
Standard 4	123 ng/mL	6593	4%	5 435
Standard 5	283 ng/mL	13 446	1%	12 288
Standard 6	652 ng/mL	19 953	6%	18 795
Standard 7	1 500 ng/mL	27 317	2%	26 158



REACH European regulations and compliance This product and/or some of its components include a Triton concentration of 0.1% or more and as such, it is concerned by the RE ACH European regulations. We recommend researchers using this product to act in compliance with REACH and in particular: to only use the product for in vitro research in appropriate and controlled premises by qualified researchers, ii) to ensure the collection and the treatment of subsequent waste, and iii) to make sure that the total amount of Triton handled does not exceed 1 ton per year



Manufactured by Cisbio Bioassays - Parc Marcel Boiteux - 30200 Codolet – FRANCE

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