MANUAL

Technology: HTRF® Biomarkers

HTRF Human Survival Motor Neuron Kits

Part number:	63ADK033PEG	63ADK033PEH
Test size	500 tests	10,000 tests

Storage: ≤ 60°C

Version: 06 **Date:** February 2024

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of Human Survival Motor Neuron in cells and offers a fast alternative to ELISA.

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

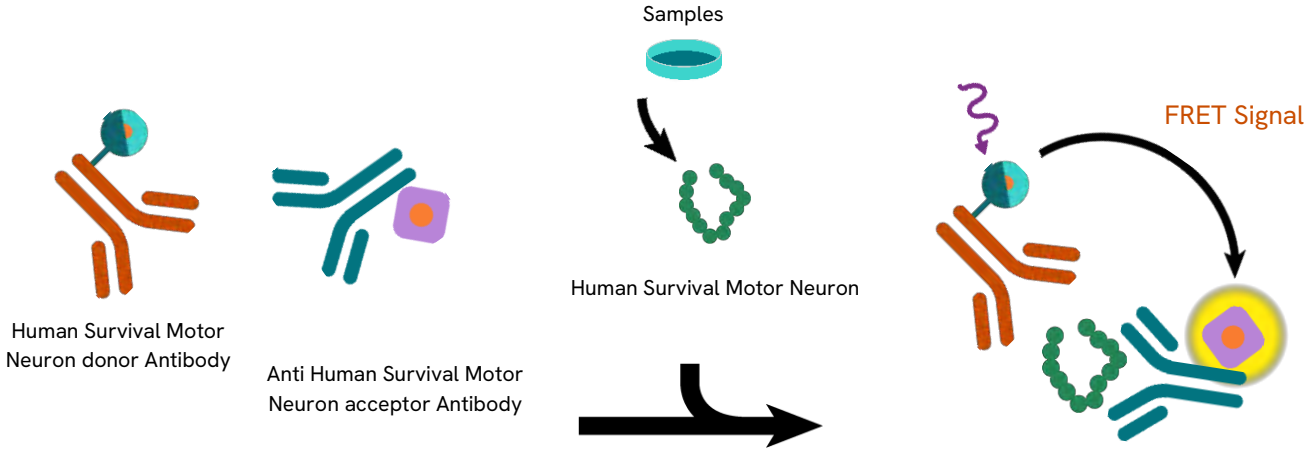
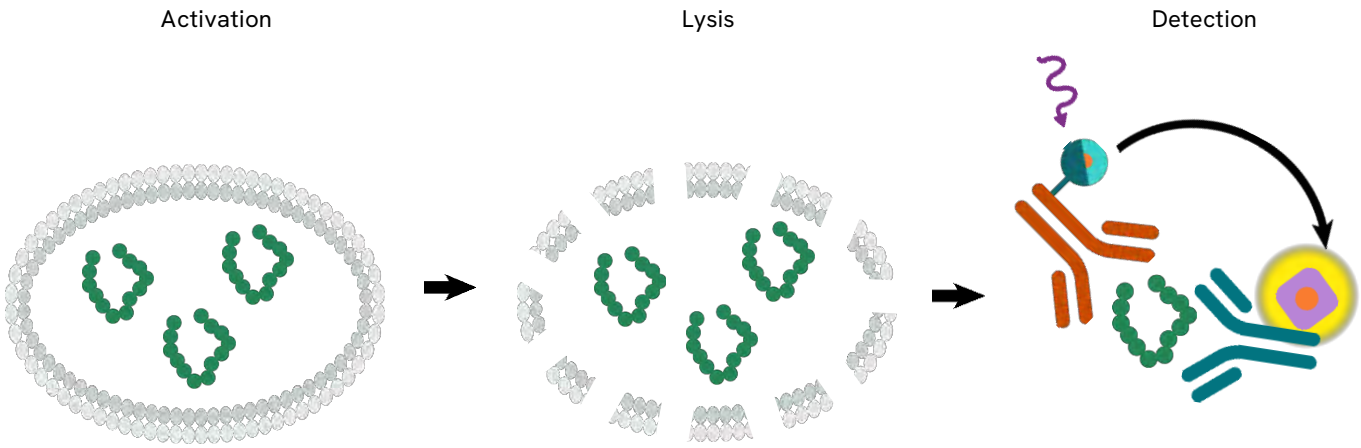
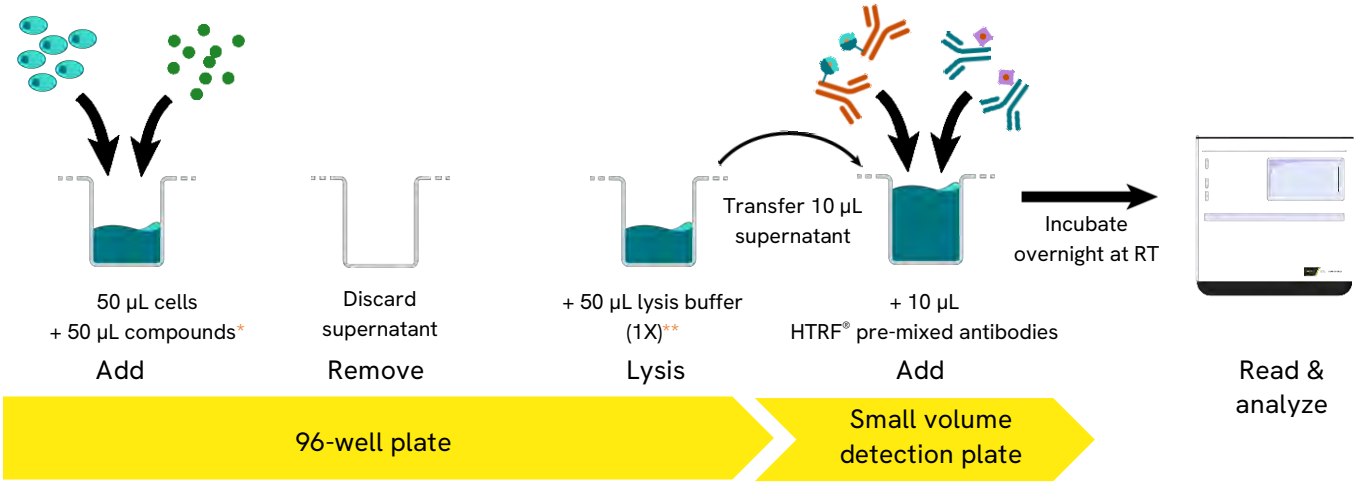


Figure 1: Principle of HTRF Human Survival Motor Neuron sandwich assay

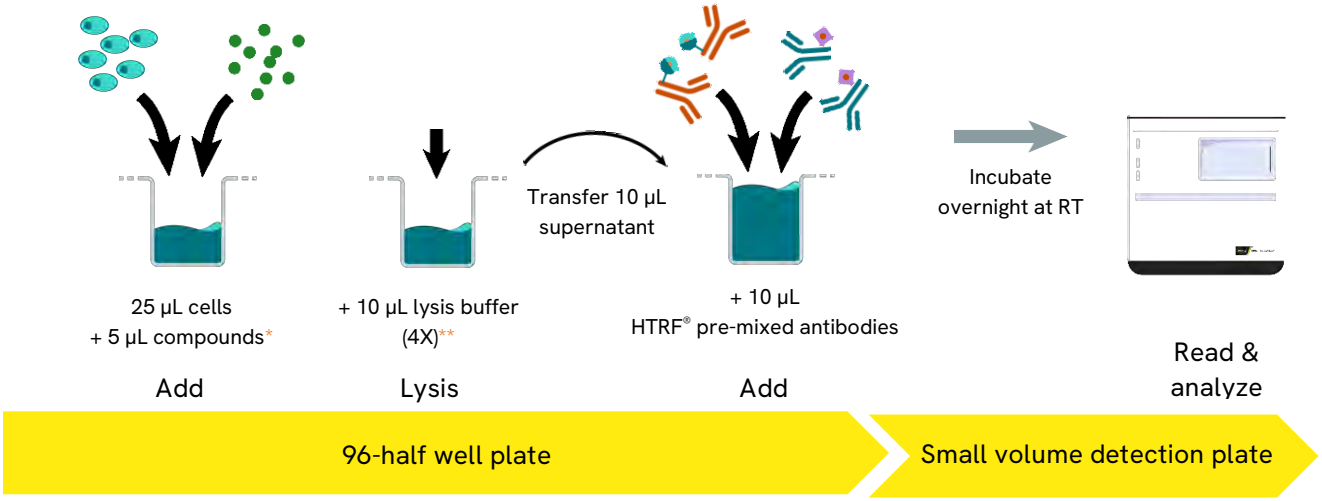
PROTOCOL AT A GLANCE



Two-plate assay protocol for adherent cells



Two-plate assay protocol for suspension cells



* Note that concentration above 0.5% DMSO will impair assay performances. Same final concentration of DMSO must be used for each compound dilutions.

** Depending on cell lines used, volume of lysis should be optimized, it can be necessary to dilute the cell lysate to ensure samples are within the assay linear range.

For HTRF certified reader

For more information about HTRF® compatible readers and for set-up recommendations, please visit our website.

MATERIAL PROVIDED

KIT COMPONENTS	500 TESTS*			10,000 TESTS*		
Control lysate (Frozen/ready-to-use)		green cap	1 vial – 150 µL		green cap	2 vials – 150 µL
Human Survival Motor Neuron Eu Cryptate Antibody (Frozen/50X)		orange cap	1 vial 50 µL		red cap	1 vial 1 mL
Human Survival Motor Neuron d2 Antibody Frozen (Frozen/50X)		blue cap	1 vial 50 µL		purple cap	1 vial 1 mL
Lysis buffer* #2 (Frozen/4X)		white cap	4 vials 2 mL		white cap	1 vial 130 mL
Detection Buffer #3 (Frozen/ready-to-use)**		red cap	1 vial 7 mL		red cap	1 vial 105 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.
** 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 our website.
- 96-well or 384-well small volume (SV) detection microplates. Use white plate only. For more information about microplate recommendations, please visit our website.

STORAGE AND STABILITY

Storage upon reception: Store the kit at -60°C or below until the expiration date indicated on the package.

Storage and stability of thawed material:

When you are ready to use the kit, take the reagents out and prepare them following the protocol provided in this document. Unused thawed reagents can be stored and conserved for future use. Refer to the table below for storage options and corresponding shelf life.

	STORAGE AFTER THAWING/RECONSTITUTION
Lysis Buffer / Blocking Reagent / Detection buffer	2-8°C until the expiration date indicated on the package
Antibodies*	2-8°C for 48h or freeze at -16°C or below until the expiration date indicated on the package for long term storage
Protein/standard/Control Lysate*	freeze at -60°C or below until the expiration date indicated on the package for long term storage

*For Antibodies, Protein, Standard & control lysate, Stock solutions may be thawed and frozen only once. Freeze in aliquots to avoid multiple freeze/thaw cycles (once aliquoted, single use of the reagent).
Volume of antibodies aliquots should not be under 10µL. Volume of Protein, Standard & control lysate aliquots should not be under 20µL.

REAGENT PREPARATION

Allow all reagents to thaw before use. We recommend centrifuging the vials gently after thawing, before pipeting the stock solutions. Prepare the working solutions from stock solutions by following the instructions below.

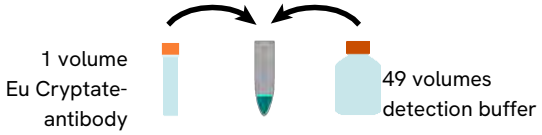
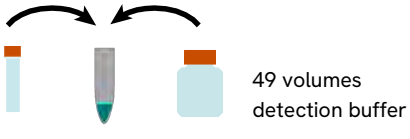
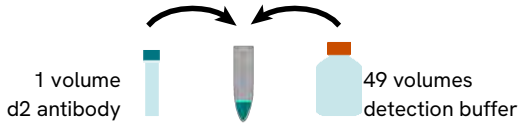
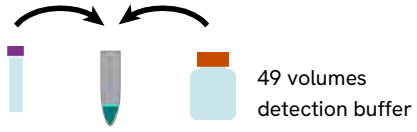
To prepare working control lysate solution

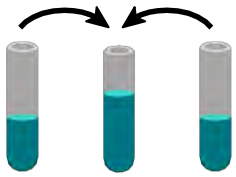
The control lysate is only provided as an internal assay control to check the quality of the results obtained. The window between control lysate and negative control should be greater than 2.
Thaw the control lysate. Mix gently, the control lysate is ready to use.

To prepare working antibody solutions

HTRF® reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of the d2 and Eu Cryptate-antibodies will impair the assay's quality. Be careful, as working solution preparation for antibodies may differ between the 500 and 10,000 tests data point kit.

Antibody working solutions are stable for 2 days at 2-8°C. Dilute the antibodies with detection buffer. In practice:

<table>
<tr><th>500 TESTS KIT</th><th>10,000 TESTS KIT</th></tr>
<tr><td colspan="2">Human Survival Motor Neuron Eu Cryptate Antibody</td></tr>
<tr><td> 
</td><td> 
</td></tr>
<tr><td>Dilute 50-fold the frozen stock solution with detection buffer e.g add 2.45 ml of detection buffer to the 0.05 ml of Eu Cryptate-antibody stock solution.</td><td>Dilute 50-fold the frozen stock solution with detection buffer e.g add 49 ml of detection buffer to the 1 ml of Eu Cryptate-antibody stock solution.</td></tr>
<tr><td colspan="2">Human Survival Motor Neuron d2 Antibody</td></tr>
<tr><td> 
</td><td> 
</td></tr>
<tr><td>Dilute 50-fold the frozen stock solution with detection buffer e.g add 2.45 ml of detection buffer to the 0.05 ml of d2-antibody stock solution.</td><td>Dilute 50-fold the frozen stock solution with detection buffer e.g add 49 mlof detection buffer to the 1 ml of d2-antibody stock solution.</td></tr>
<tr><td colspan="2">Antibody mix</td></tr>
<tr><td>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 EuCryptate-antibody solution.</td><td>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 EuCryptate-antibody solution.</td></tr>
</table>



To prepare lysis buffer

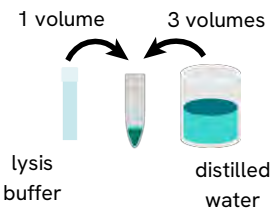
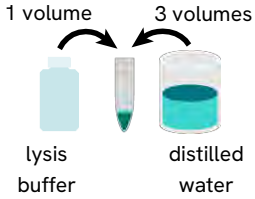
Make sure that the lysate has been generated by using the kit reagents.

Lysis buffer concentration differs between the protocols (Lysis buffer is used at 4X in the two-plate protocol on suspension cells and 1X in the two-plate protocol on adherent cells). Make sure to use the appropriate protocol's specification.

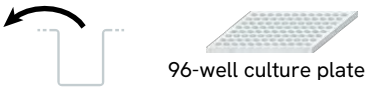
Prepare the required amount of supplement lysis buffer before running the assay, working solutions are stable for 2 days at 2-8°C.

Lysis buffer 1X for two-plate assay protocol on adherent cells

Determine the amount of lysis buffer needed for the experiment. Each well requires generally 50 µL of lysis buffer. Prepare a lysis buffer solution 1X. In practice:

TWO-PLATE ASSAY PROTOCOL ON ADHERENT CELLS	
500 TESTS KIT & 10,000 TESTS KIT	
Preparation of lysis buffer 1X	
500 tests  	10,000 tests 
Dilute the "lysis buffer 4X" 4-fold with distilled water to prepare lysis buffer 1X. e.g. take 1.25 mL of lysis buffer 4X and add it to 3.75 mL of distilled water. Mix gently.	

TWO-PLATE ASSAY PROTOCOL

	GENERAL LAB WORK PRIOR USING HTRF KIT: CELLS PREPARATION		
	FOR ADHERENT CELLS	**FOR SUSPENSION CELLS**	
1	Plate 50 µL of cells in 96-well tissue-culture treated plate in appropriate growth medium and incubate overnight, at 37°C in CO2 atmosphere.	Plate 25 µL of cells in 96 half-well plate in your appropriate medium.	 96-well culture plate
	Cell seeding densities of 100K cells/well are generally sufficient for most cell lines, but optimization of cell seeding densities is recommended. Depending on receptor a starving step with serum-free medium could be essential.		
2	Dispense 50 µL of compound (2X) diluted in cellculture serum-free medium.	Dispense 5 µL of compound (6X), diluted in yourappropriate medium.	 96-well culture plate
	For most compound, incubation time should be above 24 hours at 37°C. We recommend a time course study to determine the optimal stimulation time.		
3	Remove carefully cell supernatant either by aspirating supernatant or by flicking the plate.	Do not remove your appropriate medium.	Discard supernatant (for adherent cells)  96-well culture plate
	HUMAN SURVIVAL MOTOR NEURON DETECTION USING HTRF KIT		
	FOR ADHERENT CELLS	**FOR SUSPENSION CELLS**	
4	Immediately add 50 µL of lysis buffer(1X) and incubate for at least 30 minutes at room temperature under shaking.	Immediately add 10 µL of lysis buffer(4X) and incubate for at least 30 minutes at room temperature under shaking.	 96-well culture
	Use the appropriate lysis buffer and incubate at room temperature with shaking. Lysis incubation time may be optimized. Lysis volume can be decreased down to 25 µL.		
5	After homogenization by pipeting up and down, transfer 10 µL of cell lysate from the 96-well cell-culture plate to a small volume (SV) white detection plate. Depending on cell lines used, it can be necessary to dilute the cell lysate to ensure samples are within theassay linear range		 96-well culture SV detection plate
6	Add 10 µL of premixed antibody solutions (vol/vol) prepared in the detection buffer.Cover the plate with a plate sealer. Incubate overnight at room temperature. Set up your reader for Eu^{3+} Cryptate and read the fluorescence emission at two different wavelengths (665nm and 620nm) on a compatible HTRF® reader.		 SV detection plate

Standard protocol for two-plate assay protocol in 20µL final volume (after lysis step)

		NON TREATED CELL LYSATE	TREATED CELL LYSATE	CONTROL LYSATE	NEGATIVE CONTROL
Step 1		Dispense 10 µL of non treated cell lysate	Dispense 10 µL of treated cell lysate	Dispense 10 µL of control lysate	Dispense 10 µL of lysis buffer (1X)
Step 2		Add 10 µL of premixed Human Survival Motor Neuron antibody solution to all wells			
Step 3		Cover the plate with a plate sealer. Incubate overnight at room temperature.			
Step 4		Remove the plate sealer and read on an HTRF compatible reader			

The Negative control is used to check the non-specific signal. The ratio between control lysate signal / non-specific signal should be greater than 2.

DATA REDUCTION & INTERPRETATION

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

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

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

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

For more information about data reduction, please visit our website.

RESULTS

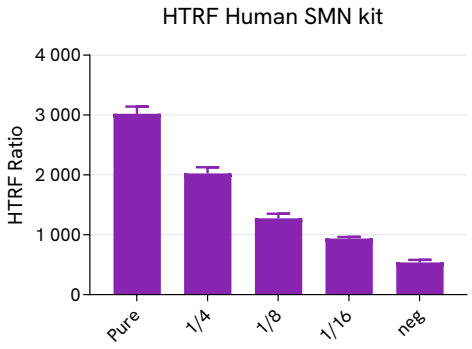
This data must not be substituted for the data obtained in the laboratory, and should be considered only as an example.

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

The graph is drawn up by plotting HTRF® Ratio versus the dilutions of the control lysate provided in the kit.

Results were obtained on an HTRF compatible reader using a flash lamp.

Serial dilutions of control lysate	HTRF Ratio (1)	CV % (2)
Pure	3022	4
1/4	2026	5
1/8	1276	6
1/16	937	3
Negative control	538	8



GENERAL LAB WORK PRIOR USING HTRF KIT: CELLS AND LYSATE PREPARATION

Frequently asked questions/troubleshooting parameters

Using adherent cells, allow time for your cells to recover after plating	Allow cells to regain full signaling capacity by plating them at least 6 hours beforestarting the pharmacological treatment.
Depending on the pathway, a serum starving step could be essential to reduce the basal level activity. This step should be optimized case-by-case.	Advice on cell culture conditions prior using HTRF® kit: For adherent cells Before treating the cells with compounds, remove culture media from the plate andreplace it with serum-free media before incubating from 2 hours up to overnight at 37°C. For suspension cells Starvation step should be carried out in the flask. Harvest cells by centrifugation and re-suspend cells at a suitable cell density in serum-free media, incubate from 2 hoursup to overnight at 37°C.
Generation of lysates	Lysates generated with HTRF® buffers can be used in other technologies, like Western-Blot. The lysis buffer is effective for creating cell extract under non denaturing conditionsfrom both plated cells and cells pelleted from suspension cultures.
Using the two-plate assay protocol, a low signal can often be improvedby adjusting lysis volumes.	In most cases, a typical adherent cell line grown in 96-well plates is readily detected ina lysis volume of 50µL. However, the lysis volume can be adjusted from 25 µL to 200µL.
Using an improper cell density can induce poor sensitivity and low signal	Check that the cell density is correct. Too high or low cell numbers can affect assayperformances.
Parameters such as cell density, stimulation time and lysis incubationtime should be optimized for each cell line used.	The assay can be used for many adherent and non-adherent cell types, including transfected cell lines and primary cells. However, the expression and phosphorylationof the readout of interscan vary from one cell line to another. Depending on the type of treatment, and the temperature, the stimulation time can vary widely. Becauseof this, we recommend a time course study to determine the optimal compound incubation time. Depending of the nature of your cells, lysis time may vary from 30' to 1h. Because ofthis, we also recommend determination of the optimal time.
Fluorescence reading	Using an inappropriate set-up may seriously impair the results. For information about HTRF® compatible readers and for set-up recommendations, please visit our website
Assaying for multiple targets from a single lysate.	The two-plate assay protocol indicates the use of 16µL of lysate per well, whereas the 96-well cell culture microplate would generate 50µL (or more) of lysate. Therefore, a typical cell lysate can be assayed for many targets, given that temporal and expression level constraints can vary from one target to another.
Batch production of cell lysates example of T175 flask	General lab work - prior using Human Survival Motor Neuron HTRF® kit: Day1: Dispense 8 million cells in T175cm2, add 25 mL of cell culture complete medium and incubate 2 days at 37°C, 5% CO2. Day3: cell stimulation Remove cell culture medium by aspiration, wash once (do not detach the cells), add 5 mL of agonist (1x) diluted in FCS free medium and incubate at 37 ° C, 5% CO2, for the optimized time. Day3: cell lysis Remove stimulation medium, wash once (do not detach the cells), add 3 ml of 1X HTRF® lysis buffer for 30 min at Room Temperature under orbital shaking. Transfer the cell lysate to a 15 mL vial, centrifuge 10 min, 2400 rcf at RT, recover cell lysate supernatant and store aliquots at -60°C or below. For long term conservation, aliquots should be stored in liquid nitrogen.

REACH European regulations and compliance

This product and/or some of its components include a NP-40 concentration of 1% or more and as such, it is concerned by the REACH 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 NP-40 handled does not exceed 1 ton per year. This product contains material of biologic origin. Use for research purposes only. Do not use in humans or for diagnostic purposes. The purchaser assumes all risk and responsibility concerning reception, handling and storage.

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

www.revvity.com

revvity

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com