MANUAL

HTRF Histamine Dynamic Detection Kit

Part # 62HTMDPET & 62HTMDPEG

Test size#: 96 tests (62HTMDPET) and 500 tests (62HTMDPEG) - assay volume: 20 µL

Revision: #04 of September 2023

Store at: 2-8°C (62HTMDPET); 2-8°C (62HTMDPEG)

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

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of Native Histamine produced by cells in buffered solution, in cell culture supernatants or plasma 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, Histamine is detected in a competitive assay by using anti histamine antibody labeled with Europium cryptate (donor), and Histamine 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). The Histamine present in the sample competes with the binding between the two HTRF detection solutions and thereby prevents FRET from occurring. The manual involves the acylation of the Histamine from the standards and samples to increase the assay sensitivity.

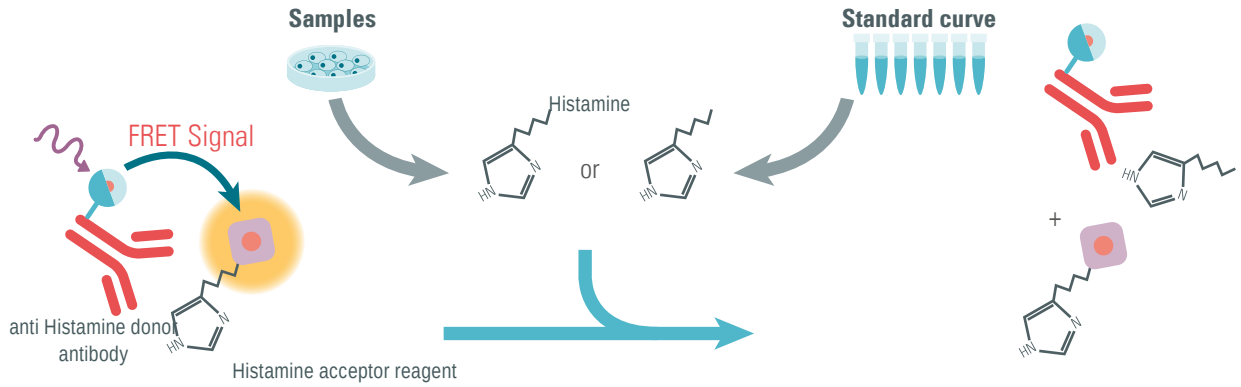
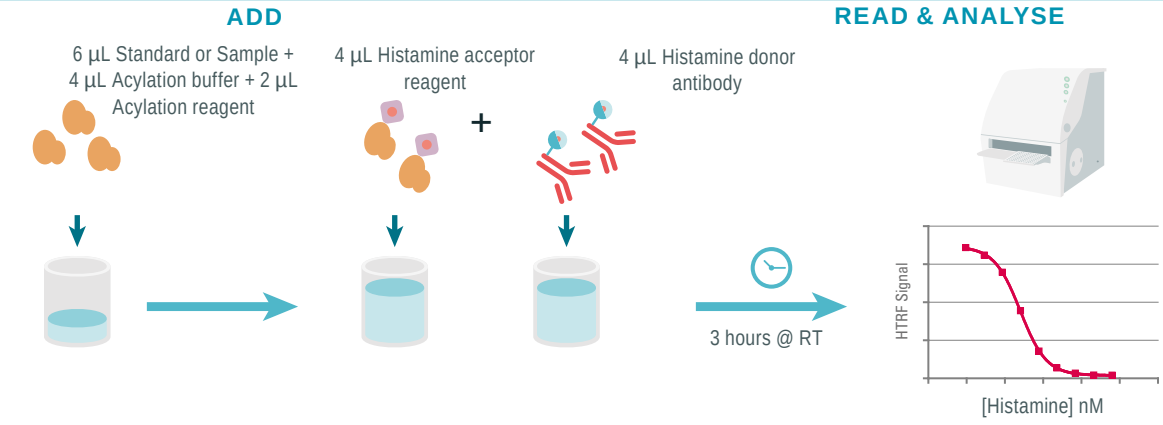


Figure 1: Principle of HTRF Histamine-Dynamic competitive assay.

MANUAL AT A GLANCE



Do not pre-mix the d2 and Cryptate solutions prior to dispensing.

Make sure to use the set-up for Eu Cryptate.

MATERIALS PROVIDED:

KIT COMPONENTS	96 TESTS * CAT # 62HTMDPET	500 TESTS * CAT # 62HTMDPEG
Histamine Standard Lyophilized	1 vial 15 µM	1 vial 15 µM
anti histamine antibody Eu Cryptate antibody	1 vial Lyophilized	1 vial Lyophilized
Histamine d2 reagent	1 vial Lyophilized	1 vial Lyophilized
Diluent or cell culture medium ** ready-to-use	1 vial 20 mL	1 vial 20 mL
Detection buffer *** ready to use	1 vial 1 mL	1 vial 5 mL
Acylation reagent Lyophilized (Solution stock 20X)	1 vial	1 vial
Acylation reagent diluent Ready-to-use	1 vial 5 mL	1 vial 5 mL
Acylation buffer Ready-to-use	1 vial 2.5 mL	1 vial 2.5 mL
DMSO	1 vial 1 mL	1 vial 1 mL
HTRF® 96-well low volume plate	1 Plate	-

* When used as advised, the two available kit sizes will provide sufficient reagents for 96 tests and 500 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.
Histamine is lyophilized in pH 7.0 buffer containing protease free BSA and stabilzers

PURCHASE SEPARATELY:

Monoclonal antibody anti-IgE Ref# MABETS07: The monoclonal anti-IgE antibody has been used as a positive control to monitor the histamine release upon treatment of blood (refer to AN: Histamine release on stimulated whole blood)

Plasma sample diluent-15 mL Ref# 62DLPDDD : The plasma sample diluent has been optimized for diluting plasma and serum samples prior to measurement with the Histamine dynamic kit

- 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 - Use white plate only..

For more information about microplate recommendations, please visit our website at: **www.revvity.com**

STORAGE AND STABILITY

Store the kit at 2-8°C.

Under proper storage conditions, reagents are stable until the expiry date indicated on the label.

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 (Can be stored 7 days at 4°C) .

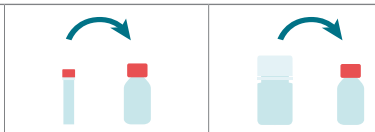
REAGENT PREPARATION

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.
- Before use, allow Diluent and Detection buffer to warm up at room temperature and homogenize them with a vortex.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- Histamine 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:

96 TESTS KIT - 62HTMDPET		500 TESTS KIT - 62HTMDPEG
anti histamine antibody Eu Cryptate antibody		
Reconstitute the anti histamine antibody Eu Cryptate antibody with 0.4 mL detection buffer. Mix gently. This ready to use 1X stock solution can be frozen and stored at -60°C or below. It can be stored unfrozen 7 days at 4°C.		Reconstitute the anti histamine antibody Eu Cryptate antibody with 2 mL detection buffer. Mix gently. This ready to use 1X stock solution can be frozen and stored at -60°C or below. It can be stored unfrozen 7 days at 4°C.
Histamine d2 reagent		
Reconstitute the Histamine d2 reagent with 0.4 mL detection buffer. Mix gently. This ready to use 1X stock solution can be frozen and stored at -60°C or below. It can be stored unfrozen 7 days at 4°C.		Reconstitute the Histamine d2 reagent with 2 mL detection buffer. Mix gently. This ready to use 1X stock solution can be frozen and stored at -60°C or below. It can be stored unfrozen 7 days at 4°C.
Histamine Standard		
Reconstitute the Histamine Standard with distilled water in order to obtain a 15 µM stock solution. See instructions on vial label for reconstitution volume. Mix gently after reconstitution. This stock solution can be stored 7 days at 4°C or may be frozen and stored at -60°C or below and thawed once only.		Reconstitute the Histamine Standard with distilled water in order to obtain a 15 µM stock solution. See instructions on vial label for reconstitution volume. Mix gently after reconstitution. This stock solution can be stored 7 days at 4°C or may be frozen and stored at -60°C or below and thawed once only.
Intermediate solution of Acylation reagent		
Reconstitute the Acylation reagent with DMSO. See instructions on vial label for reconstitution volume. Mix gently after reconstitution. Add a vortex step to make sure the acylation reagent is well resuspended. This 20 X intermediate solution can be frozen and stored at -60°C or below.		Reconstitute the Acylation reagent with DMSO. See instructions on vial label for reconstitution volume. Mix gently after reconstitution. Add a vortex step to make sure the acylation reagent is well resuspended. This 20 X intermediate solution can be frozen and stored at -60°C or below.
Detection buffer		
The Detection buffer is ready-to-use.		The Detection buffer is ready-to-use.

TO PREPARE ANTIBODY WORKING SOLUTIONS:

Each well requires 4 µL anti histamine antibody Eu Cryptate antibody and 4 µL Histamine d2 reagent.

Prepare the two solutions in separate vials.

96 TESTS KIT - 62HTMDPET		500 TESTS KIT - 62HTMDPEG
anti histamine antibody Eu Cryptate antibody		
After reconstitution, the Histamine Eu Cryptate antibody is ready to use.		After reconstitution, the Histamine Eu Cryptate antibody is ready to use.
Histamine d2 reagent		
After reconstitution, the Histamine d2 reagent is ready to use.		After reconstitution, the Histamine d2 reagent is ready to use.
Antibody mix		

TO PREPARE REAGENT STOCK SOLUTIONS:

Each well requires 2 µL of acylation reagent.

Acylation reagent		
Dilute 20-fold the intermediate solution of Acylation reagent with acylation reagent diluent: e.g. 1 volume of reconstituted reagent + 19 volumes of acylation reagent diluent. Mix gently. A cloudiness can be observed that doesn't affect the kit performances.		Dilute 20-fold the intermediate solution of Acylation reagent with acylation reagent diluent: e.g. 1 volume of reconstituted reagent + 19 volumes of acylation reagent diluent. Mix gently. A cloudiness can be observed that doesn't affect the kit performances.

TO PREPARE STANDARD WORKING SOLUTIONS:

- Each well requires 6 µL of standard.
- Dilute the standard stock solution serially with diluent or cell culture medium or in the medium used for the preparation of the samples.
- 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 or cell culture medium.
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

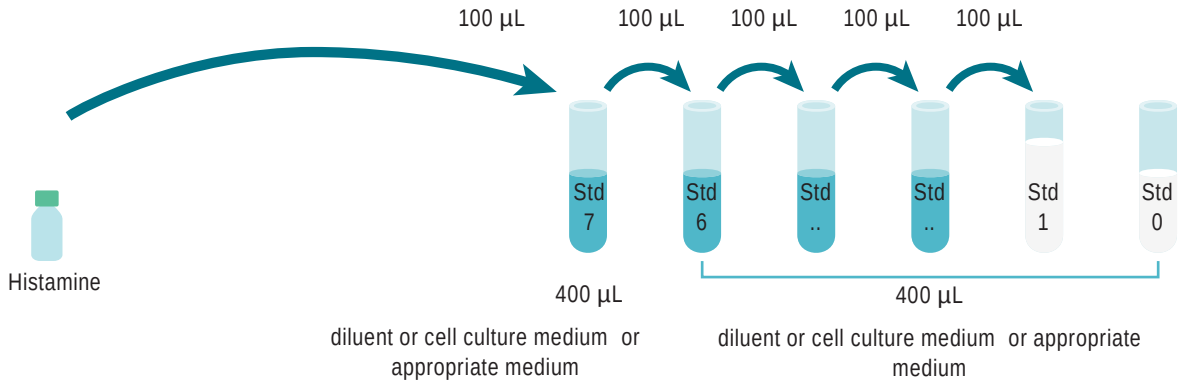
A recommended standard dilution procedure is listed and illustrated below:

Dilute the standard stock solution 5-fold with diluent or cell culture medium to prepare high standard (Std 7): e.g. take 100 µL of standard stock solution and add it to 400 µL of diluent or cell culture medium . Mix gently.

Use the high standard (Std 7) to prepare the standard curve using 1/5 serial dilutions as follows:

- Dispense 400 µL of diluent or cell culture medium in each vial from Std 6 to Std 0.
- Add 100 µL of standard to 400 µL of diluent or cell culture medium , mix gently and repeat the 1/5 serial dilution to make standard solutions: std6, std5, std4, std3, std2, std1.

This will create 7 standards for the analyte. Std 0 (Positive control) is diluent or cell culture medium or appropriate culture medium alone.



STANDARD	SERIAL DILUTIONS	HISTAMINE DYNAMIC WORKING SOLUTION (nM)
Standard Stock solution	Reconstituted lyophilisate	15,000
Standard 7	100µl standard stock solution + 400 µL Diluent	3,000
Standard 6	100 µL standard 7 + 400 µL Diluent	600
Standard 5	100 µL standard 6 + 400 µL Diluent	120
Standard 4	100 µL standard 5 + 400 µL Diluent	24
Standard 3	100 µL standard 4 + 400 µL Diluent	4.8
Standard 2	100 µL standard 3 + 400 µL Diluent	0.96
Standard 1	100 µL standard 2 + 400 µL Diluent	0.192
Standard 0	400µl Diluent	0

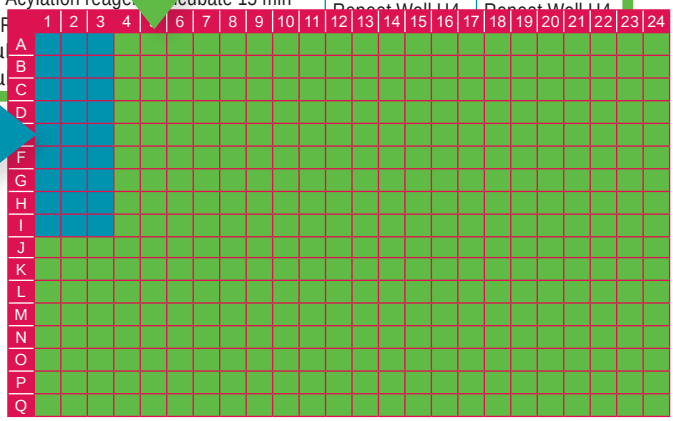
TO PREPARE SAMPLES:

- Each well requires 6 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.
- Samples with a concentration above the highest standard (Std 7) must be diluted diluent or cell culture medium or in your appropriate sample medium.

ASSAY MANUAL

	Negative control (or Cryptate control)	Standard (Std 0 - Std 7)	Sample
Step 1	Dispense 6 µL of diluent or cell culture medium into each negative well.	Dispense 6 µL of each Histamine standard (Std 0 - Std 7) into each standard well.	Dispense 6 µL of sample into each sample well.
Step 2 	4 µL acylation buffer		
Step 3 	2 µL acylation reagent working solution		
Step 4 	Seal the plate and incubate 15 min @ RT		
Step 5 	Add 4 µL of detection buffer to all wells	Add 4 µL of Histamine-d2 reagent working solution to all wells	
Step 6 	Add 4 µL of Anti-Histamine-Eu Cryptate Antibody working solution to all wells		
Step 7 	Seal the plate and incubate 3 hours @ RT		
Step 8 	Remove the plate sealer and read on an HTRF® compatible reader		

	1	2	3	4	5	6
A	6 µL diluent (Negative control) Acylation step (4 µL Acylation buffer / 2 µL Acylation reagent - incubate 15 min @ RT) 4 µL 4 µL Histamine donor antibody	Repeat Well A1	Repeat Well A1	6 µL Sample 1 Acylation step (4 µL Acylation buffer / 2 µL Acylation reagent - incubate 15 min @ RT) 4 µL Histamine acceptor reagent 4 µL Histamine donor antibody	Repeat Well A4	Repeat Well A4
B	6 µL Std 0 (Positive control) Acylation step (4 µL Acylation buffer / 2 µL Acylation reagent - incubate 15 min @ RT) 4 µL Histamine acceptor reagent 4 µL Histamine donor antibody	Repeat Well B1	Repeat Well B1	6 µL Sample 2 Acylation step (4 µL Acylation buffer / 2 µL Acylation reagent - incubate 15 min @ RT) 4 µL Histamine acceptor reagent 4 µL Histamine donor antibody	Repeat Well B4	Repeat Well B4
C	6 µL Std 1 Acylation step (4 µL Acylation buffer / 2 µL Acylation reagent - incubate 15 min @ RT) 4 µL Histamine acceptor reagent 4 µL Histamine donor antibody	Repeat Well C1	Repeat Well C1	6 µL Sample 3 Acylation step (4 µL Acylation buffer / 2 µL Acylation reagent - incubate 15 min @ RT) 4 µL Histamine acceptor reagent 4 µL Histamine donor antibody	Repeat Well C4	Repeat Well C4
D	6 µL Std 2 Acylation step (4 µL Acylation buffer / 2 µL Acylation reagent - incubate 15 min @ RT) 4 µL Histamine acceptor reagent 4 µL Histamine donor antibody	Repeat Well D1	Repeat Well D1	6 µL Sample ... Acylation step (4 µL Acylation buffer / 2 µL Acylation reagent - incubate 15 min @ RT) 4 µL Histamine acceptor reagent 4 µL Histamine donor antibody	Repeat Well D4	Repeat Well D4
E	6 µLStd ... Acylation step (4 µL Acylation buffer / 2 µL Acylation reagent - incubate 15 min @ RT) 4 µL Histamine acceptor reagent 4 µL Histamine donor antibody	Repeat Well E1	Repeat Well E1	6 µL Sample ... Acylation step (4 µL Acylation buffer / 2 µL Acylation reagent - incubate 15 min @ RT) 4 µL Histamine acceptor reagent 4 µL Histamine donor antibody	Repeat Well E4	Repeat Well E4
F	6 µL Std ... Acylation step (4 µL Acylation buffer / 2 µL Acylation reagent - incubate 15 min @ RT) 4 µL Histamine acceptor reagent 4 µL Histamine donor antibody	Repeat Well F1	Repeat Well F1	6 µL Sample ... Acylation step (4 µL Acylation buffer / 2 µL Acylation reagent - incubate 15 min @ RT) 4 µL Histamine acceptor reagent 4 µL Histamine donor antibody	Repeat Well F4	Repeat Well F4
G	6 µL Std ... Acylation step (4 µL Acylation buffer / 2 µL Acylation reagent - incubate 15 min @ RT) 4 µL Histamine acceptor reagent 4 µL Histamine donor antibody	Repeat Well G1	Repeat Well G1	6 µL Sample ... Acylation step (4 µL Acylation buffer / 2 µL Acylation reagent - incubate 15 min @ RT) 4 µL Histamine acceptor reagent 4 µL Histamine donor antibody	Repeat Well G4	Repeat Well G4
H	6 µL Std ... Acylation step (4 µL Acylation buffer / 2 µL Acylation reagent - incubate 15 min @ RT) 4 µL Histamine acceptor reagent 4 µL Histamine donor antibody	Repeat Well H1	Repeat Well H1	6 µL Sample ... Acylation step (4 µL Acylation buffer / 2 µL Acylation reagent - incubate 15 min @ RT) 4 µL Histamine acceptor reagent 4 µL Histamine donor antibody	Repeat Well H4	Repeat Well H4



DATA REDUCTION

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.

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

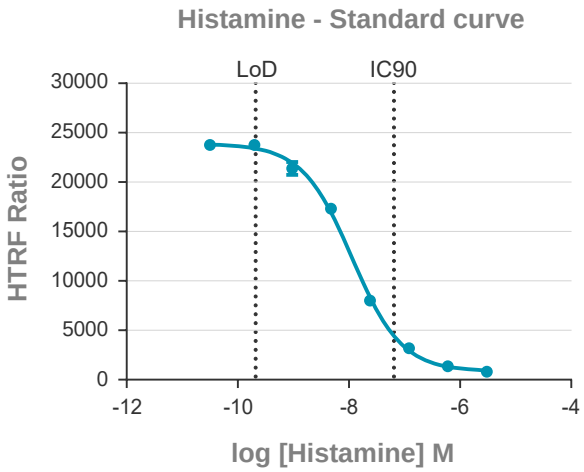
For more information about data reduction, please visit www.revvity.com

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.

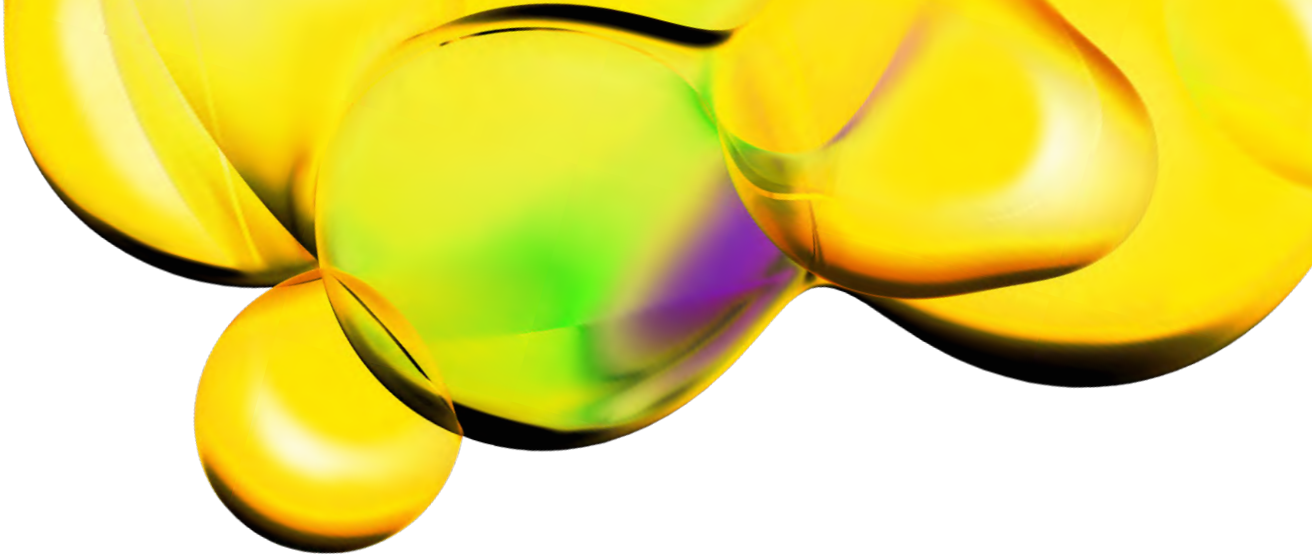
	Ratio (1)	CV (2)
Negative control	660	1.7%
Std 0 - Positive control	23,758	1.3%
Std 1 - 0.192 nM	23,754	1.3%
Std 2 - 0.96 nM	21,385	2.7%
Std 3 - 4.8 nM	17,323	2.8%
Std 4 - 24 nM	8,022	1%
Std 5 - 120 nM	3,187	2.4%
Std 6 - 600 nM	1,355	3.6%
Std 7 - 3000 nM	817	2%



ANALYTICAL CHARACTERISTICS

	Diluent
Assay range (LoD - IC90) nM	0.21 - 156
Standard curve (nM)	0.192 - 3,000
Limit of detection (LoD) = std max - 2SD	0.21nM
Incubation time	3 hours at room temperature

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.
The use of the cell line will be done with appropriate safety and handling precautions to minimize health and environmental impact.



The information provided in this document is for reference purposes only and may not be all-inclusive. Revvity, Inc., its subsidiaries, and/or affiliates (collectively, "Revvity") do not assume liability for the accuracy or completeness of the information contained herein. Users should exercise caution when handling materials as they may present unknown hazards. Revvity shall not be liable for any damages or losses resulting from handling or contact with the product, as Revvity cannot control actual methods, volumes, or conditions of use. Users are responsible for ensuring the product's suitability for their specific application. REVVITY EXPRESSLY DISCLAIMS ALL WARRANTIES, INCLUDING WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, REGARDLESS OF WHETHER ORAL OR WRITTEN, EXPRESS OR IMPLIED, ALLEGEDLY ARISING FROM ANY USAGE OF ANY TRADE OR ANY COURSE OF DEALING, IN CONNECTION WITH THE USE OF INFORMATION CONTAINED HEREIN OR THE PRODUCT ITSELF

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
Copyright ©2023, Revvity, Inc. All rights reserved.