Technology: HTRF®

Manual

Biomarkers

HTRF Active GLP-1 Kits

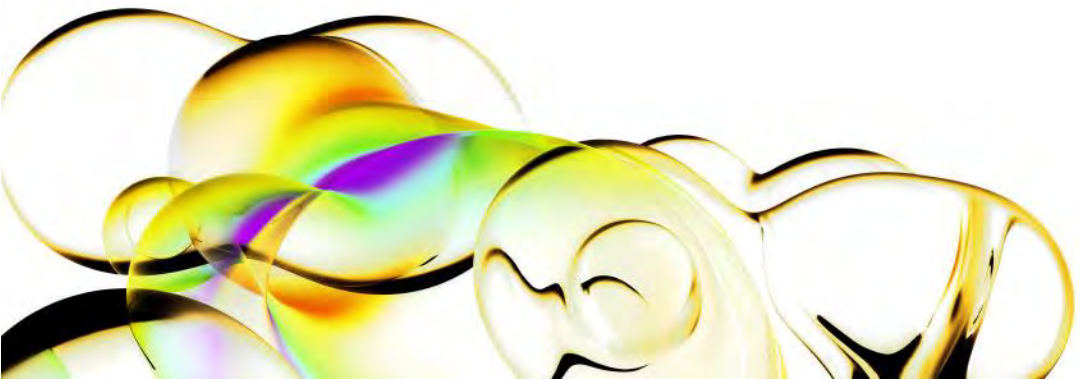
Part Numbers	62GLPPEG	62GLPPEH
Test Size	500 tests	10 000 tests

Storage: ≤-60°C or below

Assay volume: 20 µL

Version: 06

Revision date: June 2024



ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of active GLP-1 (7-36) amide & GLP-1 (7-37) in cell/tissue culture supernatants 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, Active GLP-1 is detected in a sandwich assay by using anti-active GLP-1 antibody labelled with Terbium cryptate (donor), and anti-active GLP-1 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 Active GLP-1 concentration.

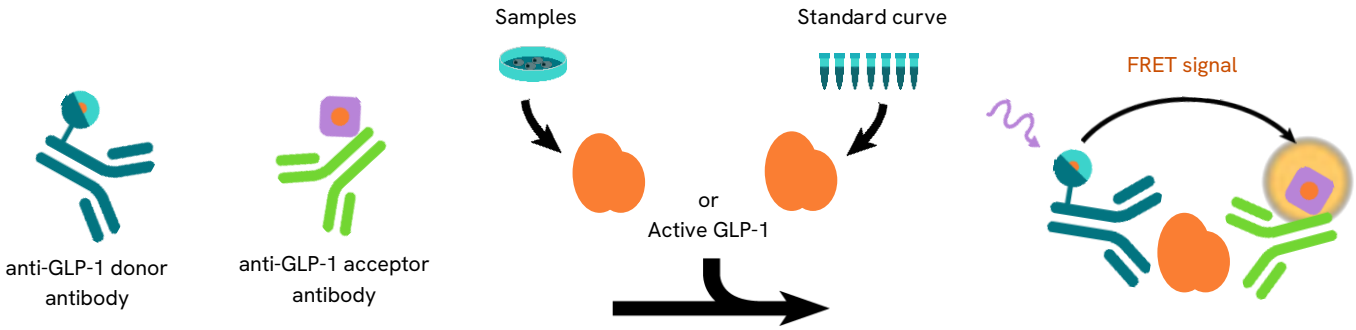
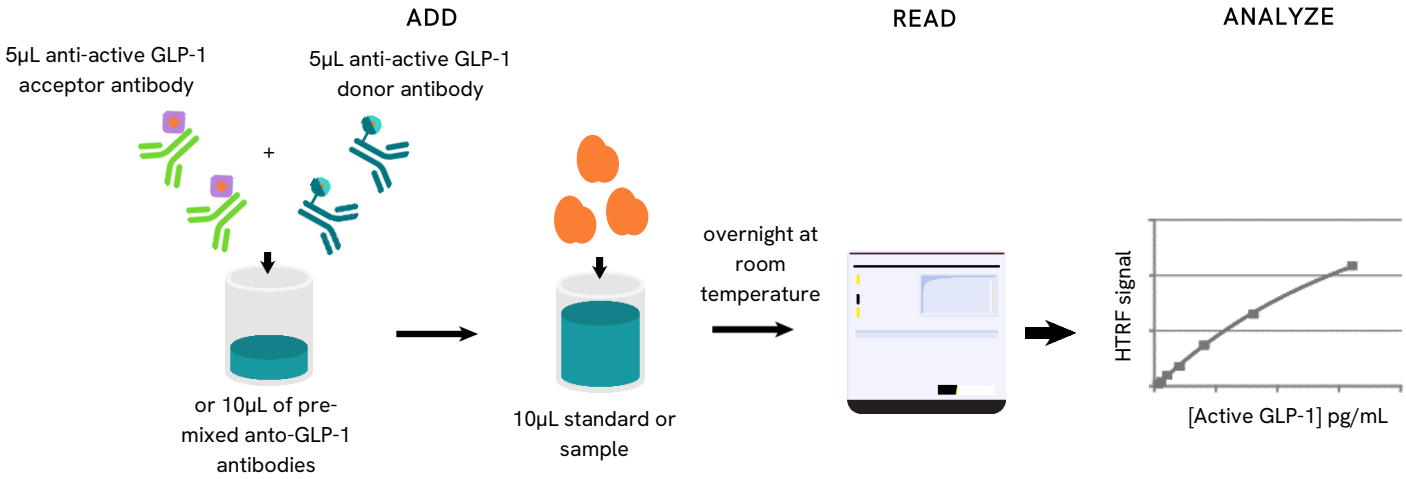


Figure 1: Principle of HTRF Active GLP-1 sandwich assay.

PROTOCOL AT A GLANCE



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

MATERIAL PROVIDED

KIT COMPONENTS	500 TESTS*	10,000 TESTS*
Active GLP-1 Standard Frozen	1 vial – 50µL 56 ng/mL	1 vial – 50µL 56 ng/mL
Active GLP-1 Tb Cryptate Antibody	1 vial - 50µL Frozen - 50X	1 vial – 1 mL Frozen - 50X
Active GLP-1 d2 Antibody	1 vial - 50µL Frozen - 50X	1 vial – 1 mL Frozen - 50X
Diluent #4 ** ready-to-use	1 vial 10 mL	1 vial 10 mL
Detection buffer #5*** ready-to-use	1 vial 7 mL	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.
** 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 our website
- Small volume (SV) detection microplates. For more information about microplate recommendations, please visit our website.

STORAGE AND STABILITY

Kit:

- Store the kit at -60°C or below.
- Under proper storage conditions, reagents are stable until the expiry date indicated on the label.

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 for standard and at -16°C or below for antibodies.
- Volume of Active GLP-1 standard aliquots should not be under 20 µL.
- Thawed diluent and detection buffer can be stored at 2-8°C in your premises

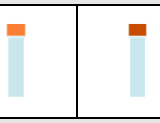
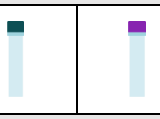
REAGENT PREPARATION

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.
- Active GLP-1 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.

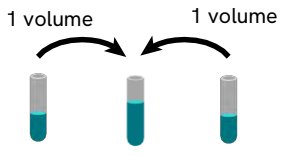
To prepare reagent stock solutions:

500 TESTS		10.000 TESTS
Anti-Active GLP-1 Tb Cryptate antibody		
Thaw the Active GLP-1 Tb Cryptate antibody. Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.		Thaw the Active GLP-1 Tb Cryptate antibody. Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.
Anti-Active GLP-1 d2 antibody		
Thaw the Active GLP-1 d2 antibody. Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.		Thaw the Active GLP-1 d2 antibody. Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.
Active GLP-1 Standard		
Thaw the GLP1 Standard in order to obtain a 56 ng/mL stock solution. Mix gently. This stock solution can be frozen and stored at -60°C or below.		Thaw the GLP1 Standard in order to obtain a 56 ng/mL stock solution. Mix gently. This stock solution can be frozen and stored at -60°C or below.
Diluent		
The Diluent is ready-to-use.		
Detection buffer		
The Detection buffer is ready-to-use.		

To prepare working solutions

Each well requires 5 µL of Active GLP-1-Tb Cryptate Antibody and 5 µL of Active GLP-1-d2 Antibody.
Prepare the two antibody solutions in separate vials.

500 TESTS	10,000 TESTS
Active GLP-1 Eu Cryptate antibody	
	
Dilute 50-fold the 50X stock solution (thawed reagent) of active GLP1 Tb Cryptate antibody with the Detection buffer #5: add 1 volume of Tb Cryptate antibody stock solution in 49 volumes of Detection buffer #5 (e.g., 50 µL of reconstituted Tb Cryptate antibody stock solution + 2.45 mL of Detection buffer #5).	Dilute 50-fold the 50X stock solution (thawed reagent) of active GLP1 Tb Cryptate antibody with the Detection buffer #5: add 1 volume of Tb Cryptate antibody stock solution in 49 volumes of Detection buffer #5(e.g., 1 mL of reconstituted Crypate antibody stock solution + 49 mL of Detection Buffer #5).
Active GLP-1 d2 antibody	
	
Dilute 50-fold the 50X stock solution (thawed reagent) of active GLP1 d2 body with the Detection buffer #5: add 1 volume of d2 body stock solution in 49 volumes of Detection buffer #5 (e.g., 50 µL of reconstituted d2 body stock solution + 2.45 mL of Detection Buffer #5).	Dilute 50-fold the 50X stock solution (thawed reagent) of active GLP1 d2 antibody with the Detection buffer #5: add 1 volume of stock solution in 49 volumes of Detection buffer #5 (e.g., 1 mL of reconstituted d2 antibody stock solution + 49 mL of Detection Buffer #5).
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 volumes of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 5 mL of d2 antibody + 5 mL of Cryptate antibody).



To prepare working standards solutions

- Each well requires 10 µL of standard.
- Dilute the standard stock solution serially with diluent #4 or with the cell culture medium used to culture the cells (e.g. KRB, KRHB, DMEM, MEM, RPMI+SVF) supplemented with 0.04% Tween 20 and 0.05% triton X-100 to avoid GLP1 sticking on tubes and tips.
- 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 #4.
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

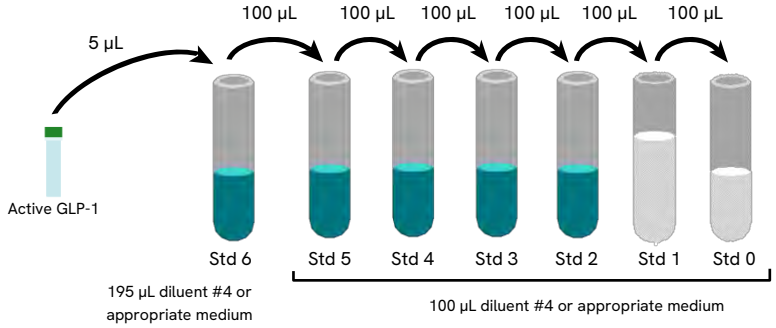
A recommended standard dilution procedure is listed and illustrated below

Dilute the standard stock solution 40-fold with diluent #4 to prepare high standard (Std 6): e.g. take 5 µL of standard stock solution and add it to 195 µL of diluent #4. Mix gently.

Use the high standard (Std 6) to prepare the standard curve using 1/2 serial dilutions as follows:

- Dispense 100 µL of diluent #4 (1X) in each vial from Std 5 to Std 0.
- Add 100 µL of standard to 100 µL of diluent #4, mix gently and repeat the 1/2 serial dilution to make standard solutions: std5, std4, std3, std2, std1.

This will create 6 standards for the analyte. Std 0 (Negative control) is diluent #4 or appropriate culture medium alone.

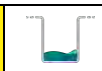


STANDARD	SERIAL DILUTIONS	WORKING SOLUTIONS
Standard Stock solution	Thawed stock solution	56,000 pg/mL
Standard 6	5 µL stock solution + 195 µL Diluent #4	1,400 pg/mL
Standard 5	100 µL standard 6 + 100 µL Diluent #4	700 pg/mL
Standard 4	100 µL standard 5 + 100 µL Diluent #4	350 pg/mL
Standard 3	100 µL standard 4 + 100 µL Diluent #4	175 pg/mL
Standard 2	100 µL standard 3 + 100 µL Diluent #4	87.5 pg/mL
Standard 1	100 µL standard 2 + 100 µL Diluent #4	43.8 pg/mL
Standard 0	100 µL Diluent #4	0

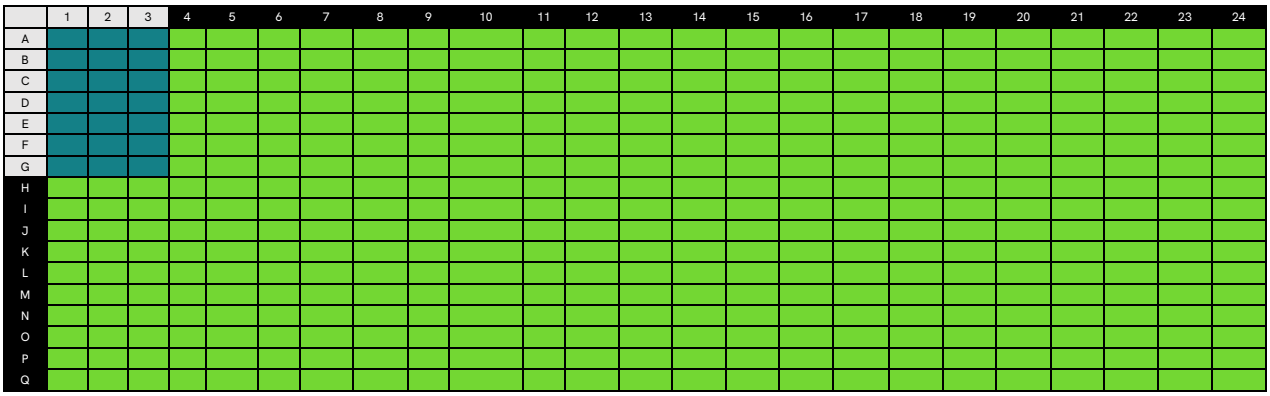
To prepare samples

- Each well requires 10 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.
- For a cell supernatant sample, we recommend the use of your own stimulation buffer without phenol red (e.g. KRB) supplemented with 0.04% tween 20 plus 0.05 % triton X-100: presence of Tween 20 plus Triton-X100 avoid sticking effect of GLP-1 during the preparation of the standard curve by serial dilutions
- Samples with a concentration above the highest standard (Std 6) must be diluted diluent #4
- The add of DPP-IV inihibitor to your stimulation buffer is highly recommended in order to avoid GLP-1 degradation (e.g. PK44 phosphate, TOCRIS Ref #4145)
- As Terbium Cryptate is sensitive to phenol red, it is mandatory to use medium without pheneol red (e.g. KRB or HSBC) to run your secretion assay.

ASSAY PROTOCOL

		STANDARD (STD 0 – STD 6)	SAMPLES
Step 1		Dispense 10 µL of each Active GLP-1 standard (Std 0 - Std 6) into each standard well	Dispense 10 µL of each sample into each sample well
Step 2		Add 5 µL of Active GLP-1 d2 antibody working solution to all wells	
Step 3		Add 5 µL of Active GLP-1 Tb Cryptate antibody working solution to all wells	
Step 4		Seal the plate and incubate overnight @ RT	
Step 5		Remove the plate sealer and read on an HTRF® compatible reader	

	1	2	3	4	5	6
A	10 µL Std 0 (Negative control) 5 µL Active GLP-1-d2 5 µL Active GLP-1-Tb Cryptate	Repeat Well A1	Repeat Well A1	10 µL sample 1 5 µL Active GLP-1-d2 5 µL Active GLP-1-Tb Cryptate	Repeat Well A4	Repeat Well A4
B	10 µL Std 1 5 µL Active GLP-1-d2 5 µL Active GLP-1-Tb Cryptate	Repeat Well B1	Repeat Well B1	10 µL sample 2 5 µL Active GLP-1-d2 5 µL Active GLP-1-Tb Cryptate	Repeat Well B4	Repeat Well B4
C	10 µL Std 2 5 µL Active GLP-1-d2 5 µL Active GLP-1-Tb Cryptate	Repeat Well C1	Repeat Well C1	10 µL sample 3 5 µL Active GLP-1-d2 5 µL Active GLP-1-Tb Cryptate	Repeat Well C4	Repeat Well C4
D	10 µL Std 3 5 µL Active GLP-1-d2 5 µL Active GLP-1-Tb Cryptate	Repeat Well D1	Repeat Well D1	10 µL sample 4 5 µL Active GLP-1-d2 5 µL Active GLP-1-Tb Cryptate	Repeat Well D4	Repeat Well D4
E	10 µL Std 4 5 µL Active GLP-1-d2 5 µL Active GLP-1-Tb Cryptate	Repeat Well E1	Repeat Well E1	10 µL sample 5 5 µL Active GLP-1-d2 5 µL Active GLP-1-Tb Cryptate	Repeat Well E4	Repeat Well E4
F	10 µL Std 5 5 µL Active GLP-1-d2 5 µL Active GLP-1-Tb Cryptate	Repeat Well F1	Repeat Well F1	10 µL sample 6 5 µL Active GLP-1-d2 5 µL Active GLP-1-Tb Cryptate	Repeat Well F4	Repeat Well F4
G	10 µL Std 6 5 µL Active GLP-1-d2 5 µL Active GLP-1-Tb Cryptate	Repeat Well G1	Repeat Well G1	10 µL sample ... 5 µL Active GLP-1-d2 5 µL Active GLP-1-Tb Cryptate	Repeat Well G4	Repeat Well G4



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 F which reflects the signal to background of the assay. The negative control (standard 0) plays the role of an internal assay control. Delta F is used for the comparison of day to day runs of the same assay.

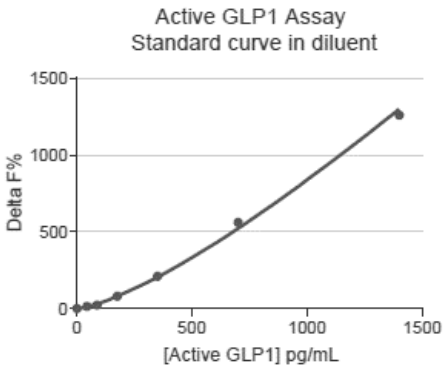
$$\text{delta F (\%)} = \frac{\text{Ratio Standard or sample - Ratio Negative Control}}{\text{Ratio Negative Control}} \times 100$$

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.
Standard curve fitting with the 4 Parameter Logistic (4PL) model:
(For more information about curve fitting please visit our website)

		Ratio (1)	CV (2)	Delta F (3)
Standard 0	Negative control	1,640	1%	-
Standard 1	43.8 pg/mL	1,876	3.9%	14%
Standard 2	87.5 pg/mL	2,024	1.6%	23%
Standard 3	175 pg/mL	2,965	1.3%	81%
Standard 4	350 pg/mL	5,089	1%	210%
Standard 5	700 pg/mL	10,828	1.5%	560%
Standard 6	1,400 pg/mL	22,290	5%	1,259%



ANALYTICAL CHARACTERISTICS

Assay performances

Assay range	43.8 to 1400 pg/mL
Limit od detection (LoD*) = Std 0 Mean + 2 SD	25 pg/mL*
Incubation time	Overnight at RT

* All HTRF compatible readers, but Spectra Max M5e and Flex Station reach this sensitivity criteria

Specificity

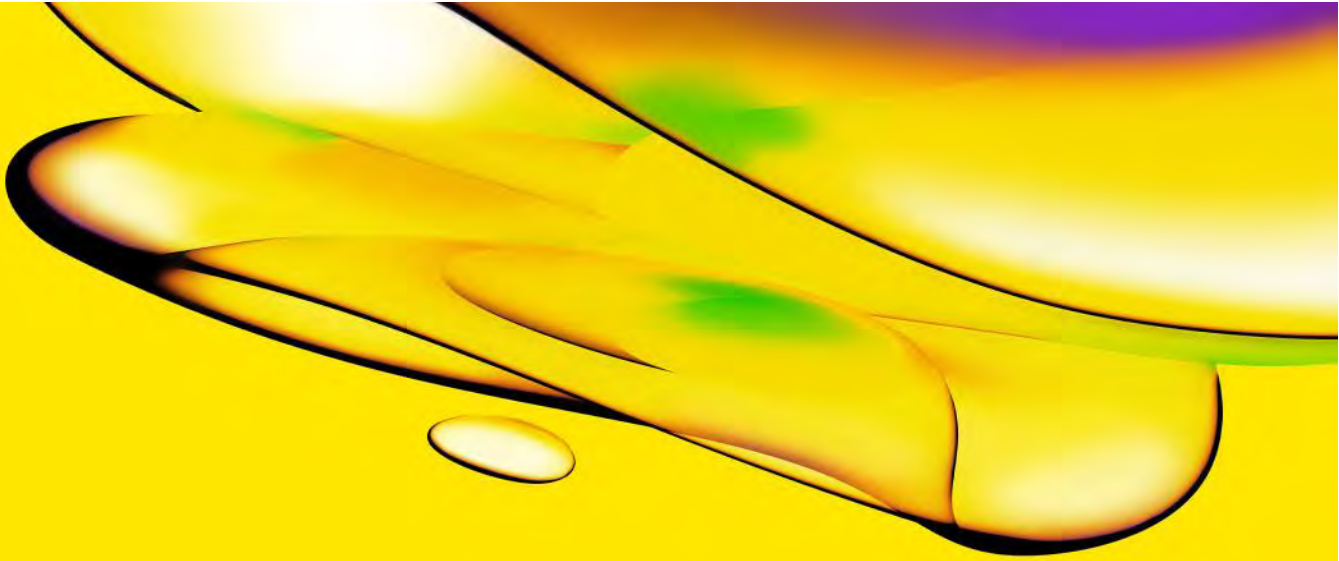
The different peptides listed below were tested up to 10 µM.

	Cross reactivity
GLP-1 (7-36) amide	100.0%
GLP-1 (7-37)	98.4%
GLP-1 (1-36) amide	0.39%
GLP-1 (1-37)	0.37%
GLP-1 (9-36) amide	<0.01%
GLP-1 (7-17)	<0.01%
GLP-2	<0.01%
Glucagon	<0.01%

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 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 Triton 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.



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