Technology: HTRF®

Manual

Protein-Protein Interaction

HTRF Human VHL Binding Kit

Part Numbers	64BDVHLPEG	64BDVHLPEH
Test Size	500 tests	10,000 tests

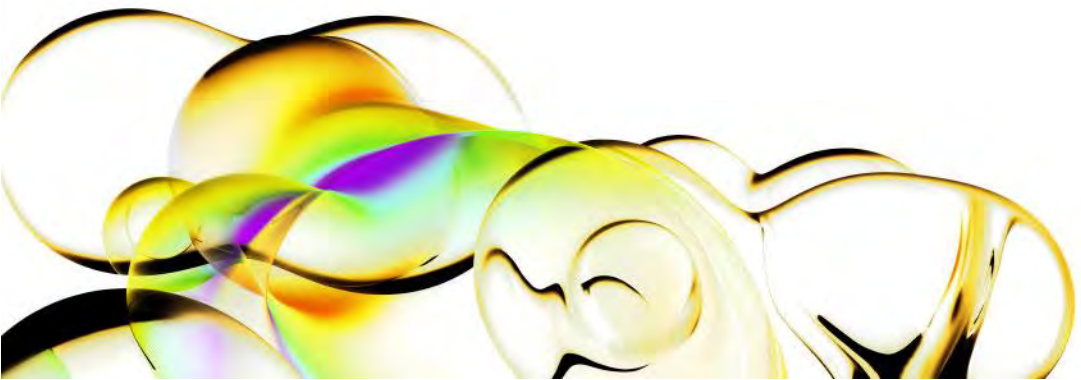
Storage: ≤-60°C

Assay volume: 20 µL

Version: 03

Revision date: June 2024

For research only. Not for use in diagnostic procedures.



ASSAY PRINCIPLE

Revvity's HTRF Human VHL Binding assay is only intended for quantitative measurement of VHL ligands using HTRF® technology.

VHL ligands are detected in a competitive assay format using a specific 6His antibody gold labeled with Europium Cryptate (donor) which binds to Human VHL 6His-tagged protein complex and HTRF VHL-Red Ligand labelled with a Red HTRF acceptor. The detection principle is based on HTRF® technology. 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). Your compound competes with the HTRF VHL-Red Ligand, and thereby prevents FRET from occurring. The specific signal is inversely proportional to the compound concentration (Fig. 1)

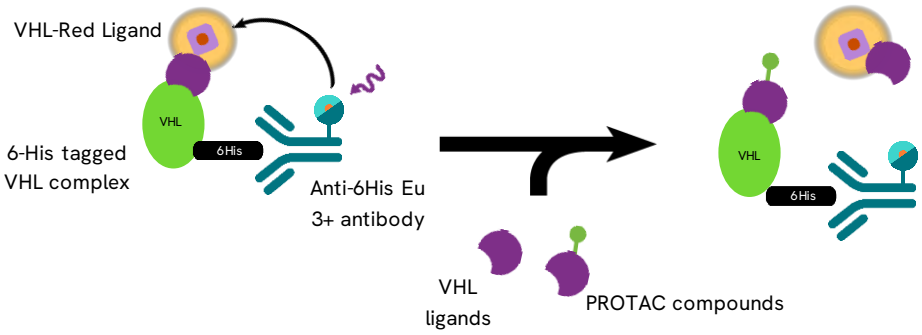
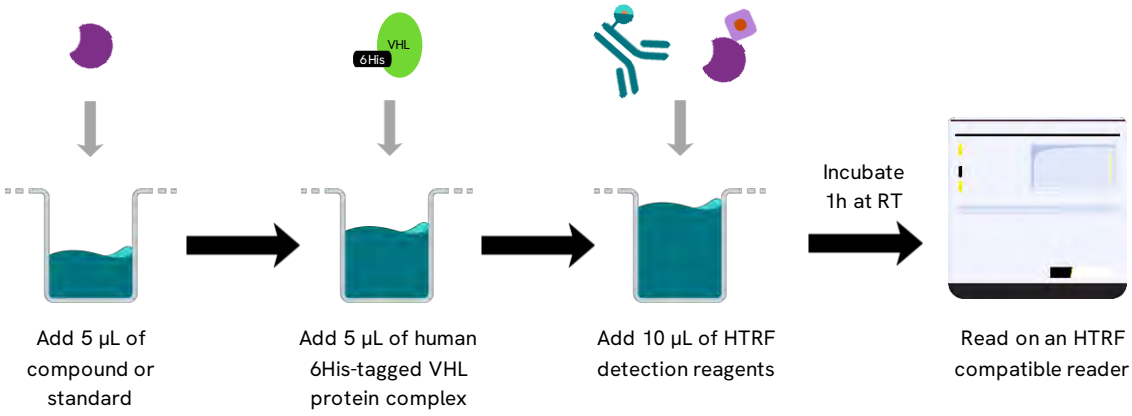


Figure 1: Principle of HTRF® Human VHL binding competitive assay.

PROTOCOL AT A GLANCE



Make sure you use the appropriate setup for Eu3+ Cryptate. For more information about setup and HTRF® compatible readers, please visit our website.

MATERIAL PROVIDED

KIT COMPONENTS	500 TESTS	10,000 TESTS
HTRF VHL Binding Kit – Standard Frozen – 5X	1 vial-50 µL	2 vials-50 µL
6His Eu Cryptate Antibody gold	1 vial-50 µL Frozen - 50X	1 vial-1 mL Frozen - 50X
HTRF VHL – Red Ligand	1 vial-50 µL Frozen - 50X	1 vial-1 mL Frozen - 50X
Human VHL 6His-tagged protein complex	1 vial-50 µL Frozen - 50X	1 vial-1 mL Frozen - 50X
Diluent #9 5X	3 vials-2 mL	1 vial- 100 mL
PROTAC binding buffer 1 1X	1 vial-20 mL	1 vial-200 mL

* When used as advised, the two available kit sizes will provide sufficient reagents for 500 and 10,000 tests respectively in 20 µL final. Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.

Purchase separately

- Low volume white (only) microplate*
- HTRF®-Certified Reader **. Make sure the setup for Eu3+ Cryptate is used.

* For HTRF microplate recommendations, please visit our website.
** For a list of HTRF-compatible readers and setup 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.

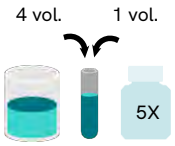
REAGENT PREPARATION

Before you begin

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent or Binding buffer may affect reagent stability and assay results.
- Thaw protein on ice, other reagents can be thawed at room temperature
- Before use, allow buffer to warm up at room temperature and homogenize it with a vortex.
- HTRF VHL Binding Kit - Standard (for standard curve) must be prepared in diluent #9. VHL binding Standard is the VH-032 compound.

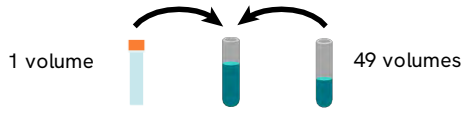
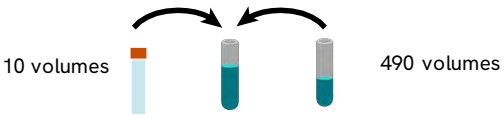
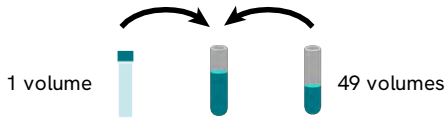
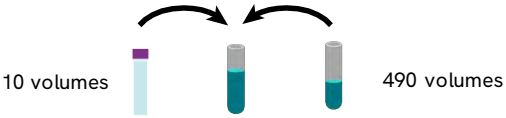
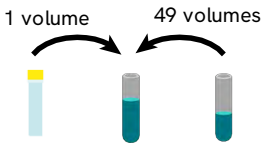
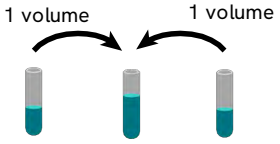
Take care to prepare stock and working solutions according to the directions for the kit size you have purchased.

To prepare reagent stock solutions

500 TESTS		10,000 TESTS
6His Eu Cryptate antibody gold		
Thaw the 6His Eu Cryptate antibody gold. Centrifuge. This 50X stock solution can be frozen and stored at -16°C or below.		Thaw the 6His Eu Cryptate antibody gold. Centrifuge. This 50X stock solution can be frozen and stored at -16°C or below.
VHL-Red Ligand		
Thaw the VHL-Red Ligand. Centrifuge. This 50X stock solution can be frozen and stored at -16°C or below.		Thaw the VHL-Red Ligand. Centrifuge. This 50X stock solution can be frozen and stored at -16°C or below
HTRF VHL Binding Kit - Standard		
Thaw the HTRF VHL Binding Kit - Standard. Centrifuge. This 5 X stock solution can be frozen and stored at -16°C or below.		Thaw the HTRF VHL Binding Kit - Standard. Centrifuge. This 5 X stock solution can be frozen and stored at -16°C or below
Human VHL 6His-tagged protein complex		
Thaw the Human VHL 6His-tagged protein complex on ice. Centrifuge the vial. To avoid freeze/thaw cycles, it is recommended to aliquot the remainder of this 50X stock solution under 20µL minimum in disposable plastic vials for storage at ≤-60°C.		Thaw the Human VHL 6His-tagged protein complex on ice. Centrifuge the vial. To avoid freeze/thaw cycles, it is recommended to aliquot the remainder of this 50X stock solution under 20µL minimum in disposable plastic vials for storage at ≤-60°C.
Diluent #9		
Dilute 5-fold the 5X diluent #9 with distilled water: Homogenize the 5X diluent #9 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.		Dilute 5-fold the 5X diluent #9 with distilled water: Homogenize the 5X diluent #9 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
PROTAC Binding Buffer 1		
Ready to use.		Ready to use.

To prepare working solutions

Each well requires 5 µL of each reagent. Prepare in separate vials.

500 TESTS	10,000 TESTS
6His Eu Cryptate antibody gold	
	
Dilute 50-fold the 50X stock solution (thawed reagent) of 6His Eu Cryptate antibody gold with PROTAC binding buffer 1 (1X), eg 1 µL of thawed Eu cryptate antibody stock solution + 49 µL of PROTAC binding buffer 1 (1X)	Dilute 50-fold the 50X stock solution (thawed reagent) of 6His Eu Cryptate antibody gold with PROTAC binding buffer 1 (1X), eg 10 µL of thawed Eu cryptate antibody stock solution + 490 µL of PROTAC binding buffer 1 (1X).
VHL-Red Ligand	
	
Dilute 50-fold the 50X stock solution (thawed reagent) of VHL-Red Ligand with PROTAC binding buffer 1 (1X), eg 1 µL of thawed VHL-Red Ligand stock solution + 49 µL of PROTAC binding buffer 1 (1X).	Dilute 50-fold the 50X stock solution (thawed reagent) of VHL-Red Ligand with PROTAC binding buffer 1 (1X), eg 10 µL of thawed VHL-Red Ligand stock solution + 490 µL of PROTAC binding buffer 1 (1X).
Human VHL 6His-tagged protein complex	
Dilute 50-fold the 50X stock solution (thawed reagent on ice) of VHL 6Histagged protein complex with PROTAC binding buffer 1 (1X), eg 10µL of thawed protein stock solution + 490µL of PROTAC binding buffer 1 (1X).	 Dilute 50-fold the 50X stock solution (thawed reagent on ice) of VHL 6Histagged protein complex with PROTAC binding buffer 1 (1X), eg 10µL of thawed protein stock solution + 490µL of PROTAC binding buffer 1 (1X).
HTRF reagents	
It is possible to pre-mix the two ready-to-use solutions just prior to dispensing the reagents by adding 1 volume of VHL-Red Ligand solution to 1 volume of 6His Eu Cryptate antibody gold solution (e.g. 1 mL of VHL-Red Ligand + 1 mL of 6His Eu Cryptate antibody gold).	 It is possible to pre-mix the two ready-to-use solutions just prior to dispensing the reagents by adding 1 volume of VHL-Red Ligand solution to 1 volume of 6His Eu Cryptate antibody gold solution (e.g. 1 mL of VHL-Red Ligand + 1 mL of 6His Eu Cryptate antibody gold).

To prepare working standards solutions

- Each well requires 5 µL of standard.
- Dilute the standard stock solution serially with diluent #9.
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

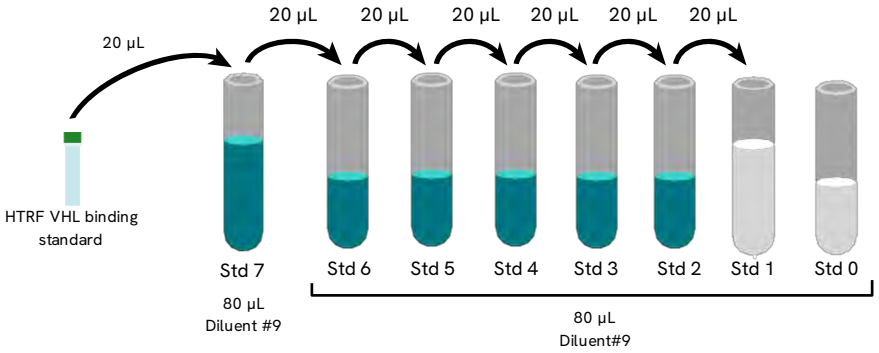
A recommended standard dilution procedure is listed and illustrated below

Dilute the standard stock solution 5-fold with diluent to prepare high standard (Std 7): take 20 µL of standard stock solution and add it to 80 µL of diluent #9 (1X). Mix gently.

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

- Dispense 80 µL of diluent #9 into each vial from Std 6 to Std 0.
- Add 20 µL of standard to 80 µL of diluent #9, 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 #9 alone



STANDARD	SERIAL DILUTIONS	STANDARD WORKING SOLUTIONS (µM)	STANDARD FINAL CONCENTRATION SOLUTIONS (µM)
Standard Stock solution	Thawed stock solution	1 000	-
Standard 7	20µl standard stock solution + 80 µL Diluent #9 (1X)	200	50
Standard 6	20 µL standard 7 + 80 µL Diluent #9 (1X)	40	10
Standard 5	20 µL standard 6 + 80 µL Diluent #9 (1X)	8	2
Standard 4	20 µL standard 5 + 80 µL Diluent #9 (1X)	1.6	0.4
Standard 3	20 µL standard 4 + 80 µL Diluent #9 (1X)	0.32	0.08
Standard 2	20 µL standard 3 + 80 µL Diluent #9 (1X)	0.064	0.016
Standard 1	20 µL standard 2 + 80 µL Diluent #9 (1X)	0.0128	0.0032
Standard 0	80 µL Diluent #9 (1X)	0	0

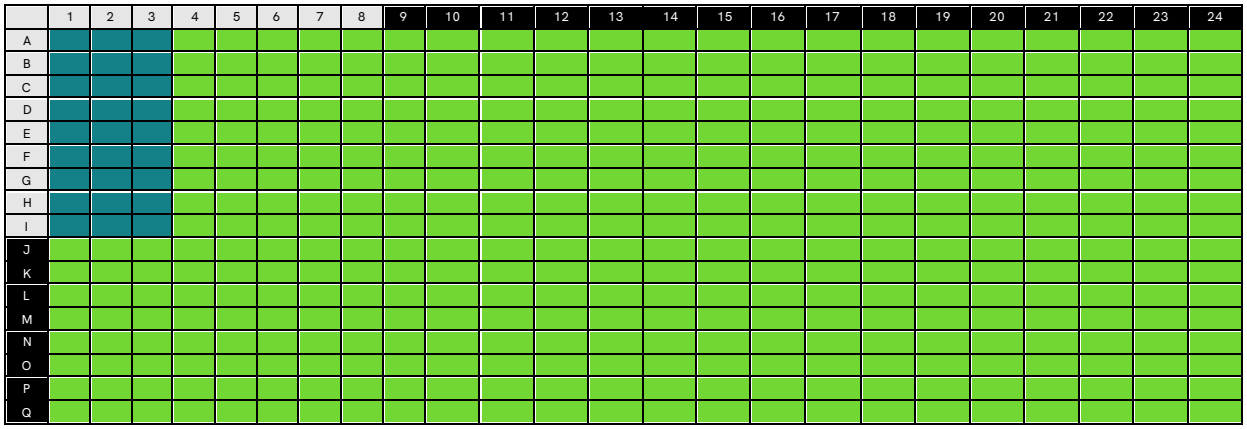
To prepare samples

- Each well requires 5 µL of compound.
- Dilute your compound in diluent #9 (1X).
- DMSO concentration must not exceed 2% final in the well (fold of change is impaired by increasing percentage of DMSO).

ASSAY PROTOCOL

		NEGATIVE CONTROL OR CYPTATE CONTROL	STANDARD (STD 0 – STD 7)	SAMPLES
Step 1		Dispense 5 µL of diluent #9 into each negative control well.	Dispense 5 µL of each HTRF VHL Binding Kit - Standard (Std 0 - Std 7) into each standard well.	Dispense 5 µL of compound into each compound well.
Step 2		Add 5 µL of PROTAC Binding buffer 1 to all wells	Add 5 µL of Human VHL 6His-tagged protein complex to all wells	
Step 3		Add 10 µL of premixed HTRF VHL-Red Ligand and 6His Eu Cryptate antibody gold working solution to all wells		
Step 4		Seal the plate and incubate 1 hour at RT		
Step 5		Remove the plate sealer and read on an HTRF® compatible reader		

	1	2	3	4	5	6
A	(Negative control) 5 µL of diluent #9 5 µL of PROTAC Binding buffer 1 10 µL of VHL-Red Ligand and 6His Eu antibody gold premixed	Repeat Well A1	Repeat Well A1	5 µL Compound 1 5 µL of VHL 6His-tagged protein complex 10 µL of VHL-Red Ligand and 6His Eu antibody gold premixed	Repeat Well A4	Repeat Well A4
B	5 µL Std 0 5 µL of VHL 6His-tagged protein complex 10 µL of VHL-Red Ligand and 6His Eu antibody gold premixed	Repeat Well B1	Repeat Well B1	5 µL Compound 2 5 µL of VHL 6His-tagged protein complex 10 µL of VHL-Red Ligand and 6His Eu antibody gold premixed	Repeat Well B4	Repeat Well B4
C	5 µL Std 1 5 µL of VHL 6His-tagged protein complex 10 µL of VHL-Red Ligand and 6His Eu antibody gold premixed	Repeat Well C1	Repeat Well C1	5 µL Compound 3 5 µL of VHL 6His-tagged protein complex 10 µL of VHL-Red Ligand and 6His Eu antibody gold premixed	Repeat Well C4	Repeat Well C4
D	5 µL Std 2 5 µL of VHL 6His-tagged protein complex 10 µL of VHL-Red Ligand and 6His Eu antibody gold premixed	Repeat Well D1	Repeat Well D1	5 µL Compound... 5 µL of VHL 6His-tagged protein complex 10 µL of VHL-Red Ligand and 6His Eu antibody gold premixed	Repeat Well D4	Repeat Well D4
E	5 µL Std ... 5 µL of VHL 6His-tagged protein complex 10 µL of VHL-Red Ligand and 6His Eu antibody gold premixed	Repeat Well E1	Repeat Well E1	5 µL Compound... 5 µL of VHL 6His-tagged protein complex 10 µL of VHL-Red Ligand and 6His Eu antibody gold premixed	Repeat Well E4	Repeat Well E4
F	5 µL Std... 5 µL of VHL 6His-tagged protein complex 10 µL of VHL-Red Ligand and 6His Eu antibody gold premixed	Repeat Well F1	Repeat Well F1	5 µL Compound... 5 µL of VHL 6His-tagged protein complex 10 µL of VHL-Red Ligand and 6His Eu antibody gold premixed	Repeat Well F4	Repeat Well F4
G	5 µL Std... 5 µL of VHL 6His-tagged protein complex 10 µL of VHL-Red Ligand and 6His Eu antibody gold premixed	Repeat Well G1	Repeat Well G1	5 µL Compound... 5 µL of VHL 6His-tagged protein complex 10 µL of VHL-Red Ligand and 6His Eu antibody gold premixed	Repeat Well G4	Repeat Well G4
H	5 µL Std... 5 µL of VHL 6His-tagged protein complex 10 µL of VHL-Red Ligand and 6His Eu antibody gold premixed	Repeat Well H1	Repeat Well H1	5 µL Compound... 5 µL of VHL 6His-tagged protein complex 10 µL of VHL-Red Ligand and 6His Eu antibody gold premixed	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.

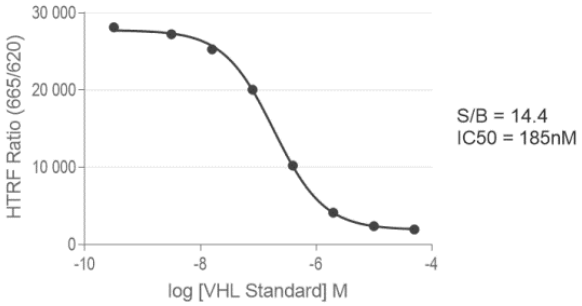
$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

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 (readouts on an HTRF compatible reader). Results may vary from one HTRF® compatible reader to another.

		Ratio (1)	CV% (2)
Negative control		1 896	2.0%
Standard 0		28 145	1%
Standard 1	0.0032 µM	27 235	2%
Standard 2	0.016 µM	25 291	2%
Standard 3	0.08 µM	20 061	0%
Standard 4	0.4 µM	10 229	0%
Standard 5	2 µM	4 127	2%
Standard 6	10 µM	2 398	2%
Standard 7	50 µM	1 952	3%



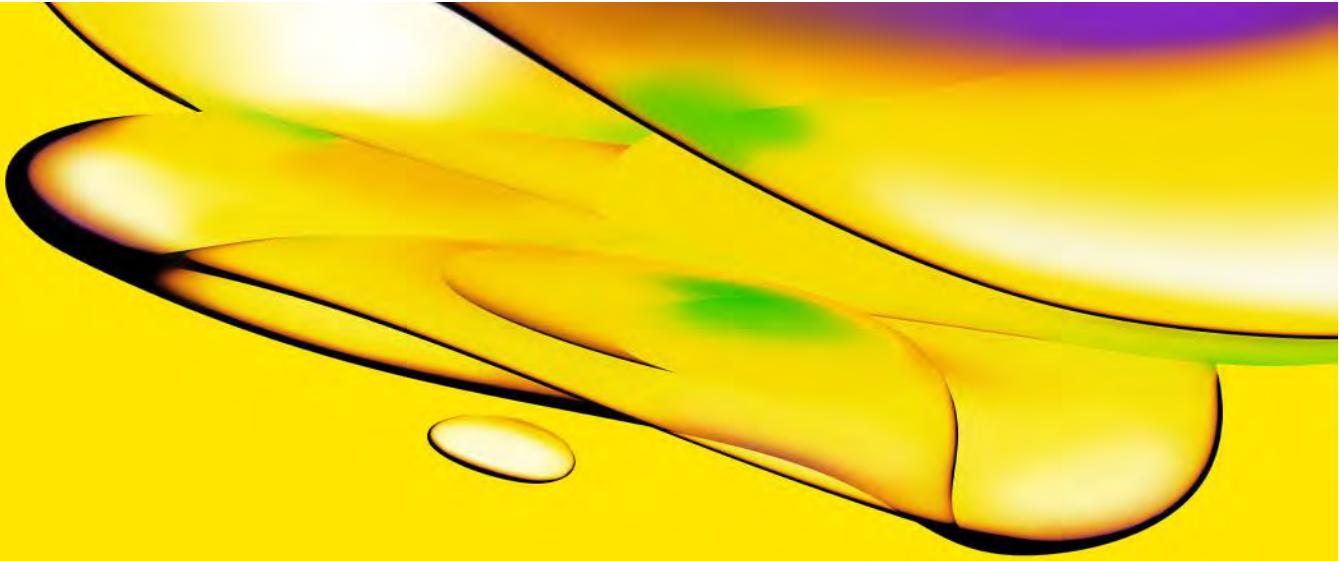
ANALYTICAL CHARACTERISTICS

HTRF VHL-Red Ligand Kd	35 nM
HTRF VHL-Red Ligand concentration	20 nM
HTRF VHL Binding Kit - Standard IC50	185 nM
HTRF VHL Binding Kit - Standard Ki	108 nM

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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