MANUAL

Technology: HTRF® Biomarkers

HTRF AAV5 Capsid detection Kit

Part number	64AAV5PEG	64AAV5PEH
Test size	500 tests	10,000 tests

Storage: ≤-60°C or below

Version: 02 **Date:** March 2024

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of Adeno-associated virus serotype 5 (AAV5) particles (being expressed in Viral Particles/mL or VP/mL) in both cell lysates and supernatants and offers a fast alternative to ELISA.

AAVs are used as vector for gene delivery mainly because of their ability to infect cells without pathogenicity, and its ease of engineering and production. AAV5 is an efficient vector for lung, retina or central nervous system.

The detection principle of this kit is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1, AAV5 capsid is detected in a sandwich assay by using a pre-mixture of biotinylated anti-AAV5 antibody bound to the streptavidin Europium cryptate (donor), and anti-AAV5 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 AAV5 capsid concentration.

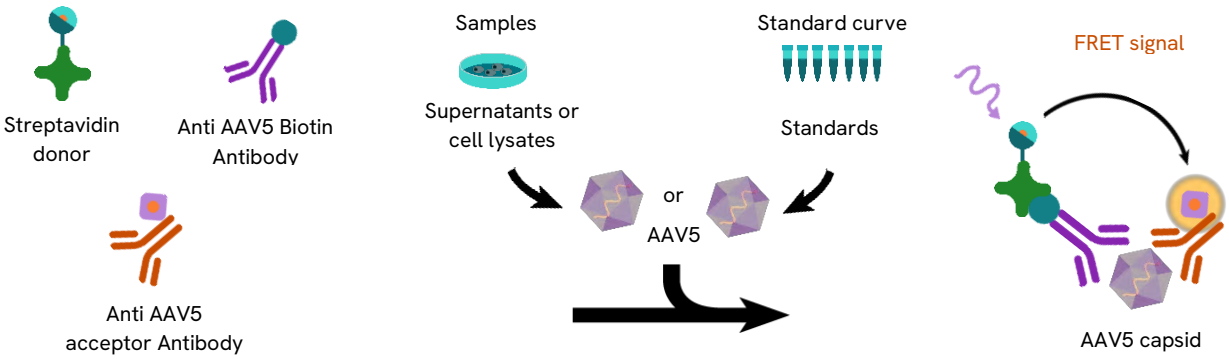
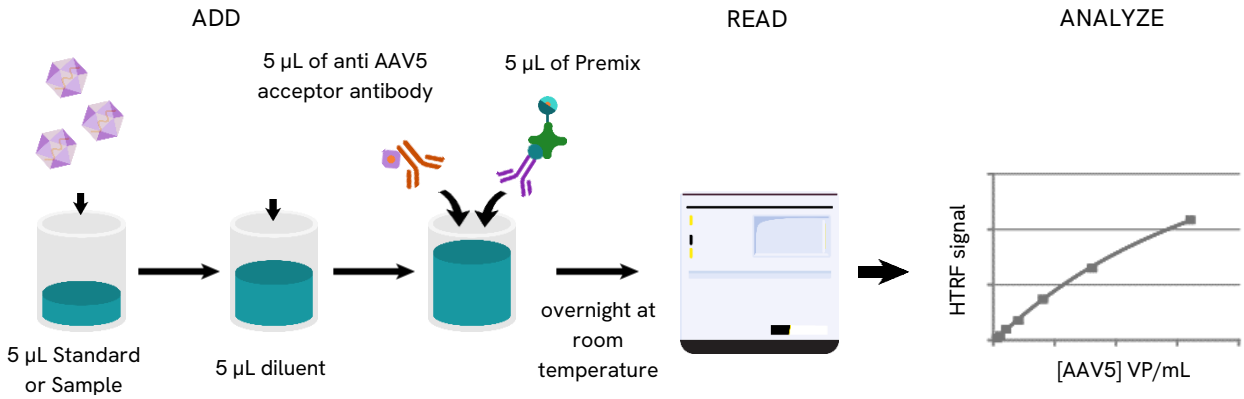


Figure 1: Principle of HTRF AAV5 sandwich assay.

PROTOCOL AT A GLANCE



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

MATERIAL PROVIDED

KIT COMPONENTS	500 TESTS*			10,000 TESTS*		
AAV5 Standard Frozen		green cap	1 vial 5.00E+11 VP/mL		green cap	2 vials 5.00E+11 VP/mL
Eu Cryptate Streptavidin 20X		orange cap	1 vial 65 µL		red cap	1 vial 1.25 mL
AAV5 Biotin Antibody 20X		orange cap	1 vial 65 µL		red cap	1 vial 1.25 mL
AAV5 d2 Antibody 20X		blue cap	1 vial 125 µL		purple cap	2 vials 1.25 mL
Diluent** #5 5X		yellow cap	1 vial 2 mL		white cap	1 vial 100 mL
Detection Buffer*** #3 Ready-to-use		transparent cap	1 vial 7 mL		red cap	1 vial 105 mL
Lysis Buffer **** #3 4X		transparent cap	1 vial 2 mL		transparent cap	1 vial 2 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.
**** The Lysis buffer is used to lysate or dilute samples.

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.
- Diluent and detection buffer are shipped frozen but can be stored at 2-8°C in your premises.

Reagents:

- Once thawed, antibody and standard solutions can be frozen once.
- To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at ≤-60°C.
- Volume of standard and antibody aliquots should not be under 10 µL.
- Thawed diluent and detection buffer can be stored at 2-8°C in your premises

REAGENT PREPARATION

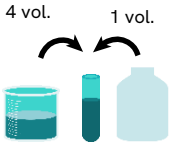
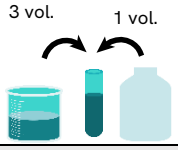
Before you begin

- 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 and premix must be prepared in individual vials.

- AAV5 standards (for standard curve) must be prepared in diluent or in the same medium as the samples (cell culture medium or lysis buffer).

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

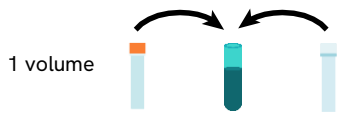
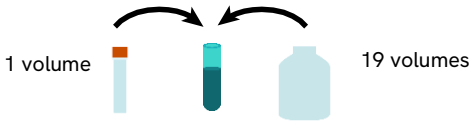
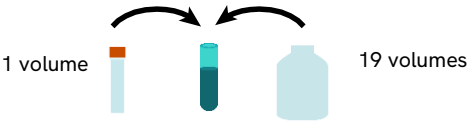
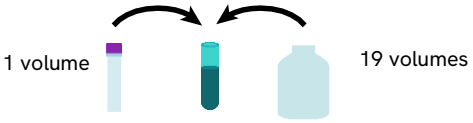
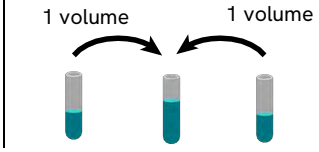
To prepare reagent stock solutions

500 TESTS			10.000 TESTS
Eu Cryptate Streptavidin			
Thaw the Eu Cryptate Streptavidin. Mix gently. This 20X 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 Eu Cryptate Streptavidin. Mix gently. This 20X 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 AAV5 Biotin antibody			
Thaw the AAV5 Biotin antibody. Mix gently. This 20X 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 AAV5 Biotin antibody. Mix gently. This 20X 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 AAV5 d2 antibody			
Thaw the AAV5 d2 antibody. Mix gently. This 20X 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 AAV5 d2 antibody. Mix gently. This 20X 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.
AAV5 capsid Standard			
Thaw the AAV5 standard to obtain 5.00E+11 VP/mL stock solution. Mix gently. 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 AAV5 standard to obtain 5.00E+11 VP/mL stock solution. Mix gently. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions 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., 20 mL of diluent + 80 mL of distilled water). Mix gently after dilution. This 1X diluent can be frozen and stored at -60°C or below.
Lysis buffer			
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.			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.
Detection buffer			

The Detection buffer is ready-to-use.

To prepare working solutions

Each well requires 5 µL of Anti AAV5 d2 antibody and 5 µL of Premix (Eu Cryptate Streptavidin + AAV5 Biotin antibody). Prepare the antibody solutions in separate vials.

500 TESTS	10,000 TESTS
Eu Cryptate Streptavidin	
	
Dilute 20-fold the 20X stock solution (thawed reagent) of Eu Cryptate Streptavidin with Detection buffer #3: add 1 volume of Eu Cryptate Streptavidin stock solution in 19 volumes of detection buffer (e.g. 65 µL of Eu Cryptate Streptavidin stock solution + 1235 µL of detection buffer).	Dilute 20-fold the 20X stock solution (thawed reagent of Eu Cryptate Streptavidin with Detection buffer #3: add 1 volume of Eu Cryptate Streptavidin stock solution in 19 volumes of detection buffer (e.g. 1.25 mL of Eu Cryptate Streptavidin stock solution + 23.75 mL of detection buffer).
AAV5 Biotin antibody	
	
Dilute 20-fold the 20X stock solution (thawed reagent) of AAV5 Biotin antibody with Detection buffer #3: add 1 volume of AAV5 Biotin antibody stock solution in 19 volumes of detection buffer (e.g. 65 µL of AAV5 Biotin antibody stock solution + 1235 µL of detection buffer).	Dilute 20-fold the 20X stock solution (thawed reagent) of AAV5 Biotin antibody with Detection buffer #3: add 1 volume of AAV5 Biotin antibody stock solution in 19 volumes of detection buffer (e.g. 1.25 mL of AAV5 Biotin antibody stock solution + 23.75 mL of detection buffer).
AAV5 d2 antibody	
	
Dilute 20-fold the 20X stock solution (thawed reagent) of AAV5 d2 antibody with Detection buffer #3: add 1 volume of d2 antibody stock solution in 19 volumes of detection buffer (e.g. 125 µL of d2 antibody stock solution + 2375 µL of detection buffer).	Dilute 20-fold the 20X stock solution (thawed reagent) of AAV5 d2 antibody with Detection buffer #3: add 1 volume of d2 antibody stock solution in 19 volumes of detection buffer (e.g. 2.5 mL of d2 antibody stock solution + 47.5 mL of detection buffer).
Premix with Eu Cryptate Streptavidin & AAV5 Biotin antibody	
Pre-mix the two ready-to-use solutions just prior to dispensing the reagents by adding 1 volume of Eu Cryptate Streptavidin solution to 1 volume of AAV5 Biotin antibody solution (e.g. 1.3 mL of Eu Cryptate Streptavidin + 1.3 mL of AAV5 Biotin antibody).	 Pre-mix the two ready-to-use solutions just prior to dispensing the reagents by adding 1 volume of Eu Cryptate Streptavidin solution to 1 volume of AAV5 Biotin antibody solution (e.g. 25 mL of Eu Cryptate Streptavidin + 25 mL of AAV5 Biotin antibody).

To prepare working standards solutions

- Each well requires 5 µL of standard.
- Dilute the standard stock solution serially with diluent #5 (1X) or with the cell culture medium or lysis buffer used to prepare your 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 or lysis buffer and in diluent #5 (1X).

- In order to counteract any standard sticking, we recommend changing tips between each dilution.

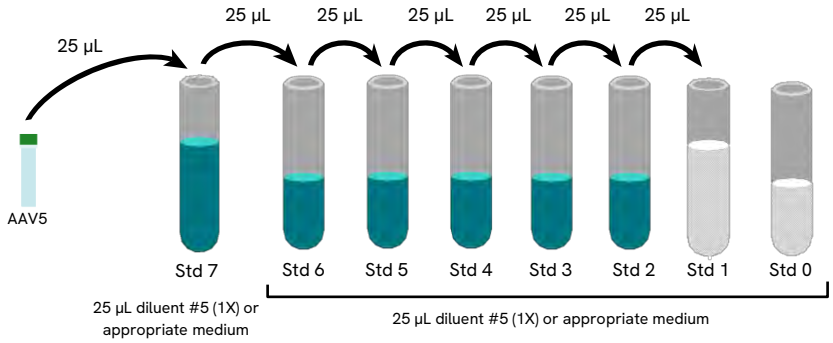
A recommended standard dilution procedure is listed and illustrated below

Dilute the standard stock solution 2-fold with diluent #5 (1X) to prepare high standard (Std 7): e.g. take 25 µL of standard stock solution and add it to 25 µL of diluent #5 (1X). Mix gently.

Use the high standard (Std 7) to prepare the standard curve using 1/2 serial dilutions as follows:

- Dispense 25 µL of diluent #5 (1X) in each vial from Std 6 to Std 0.
- Add 25 µL of standard to 25 µL of diluent #5 (1X), mix gently and repeat the 1/2 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	Thawed stock solution	5.00E+11 VP/mL
Standard 7	25 µL stock solution + 25 µL Diluent #5 (1X)	2.50E+11 VP/mL
Standard 6	25 µL standard 7 + 25 µL Diluent #5 (1X)	1.25E+11 VP/mL
Standard 5	25 µL standard 6 + 25 µL Diluent #5 (1X)	6.25E+10 VP/mL
Standard 4	25 µL standard 5 + 25 µL Diluent #5 (1X)	3.13E+10 VP/mL
Standard 3	25 µL standard 4 + 25 µL Diluent #5 (1X)	1.56E+10 VP/mL
Standard 2	25 µL standard 3 + 25 µL Diluent #5 (1X)	7.81E+09 VP/mL
Standard 1	25 µL standard 2 + 25 µL Diluent #5 (1X)	3.91E+09 VP/mL
Standard 0	25 µL Diluent #5 (1X)	0

To prepare samples

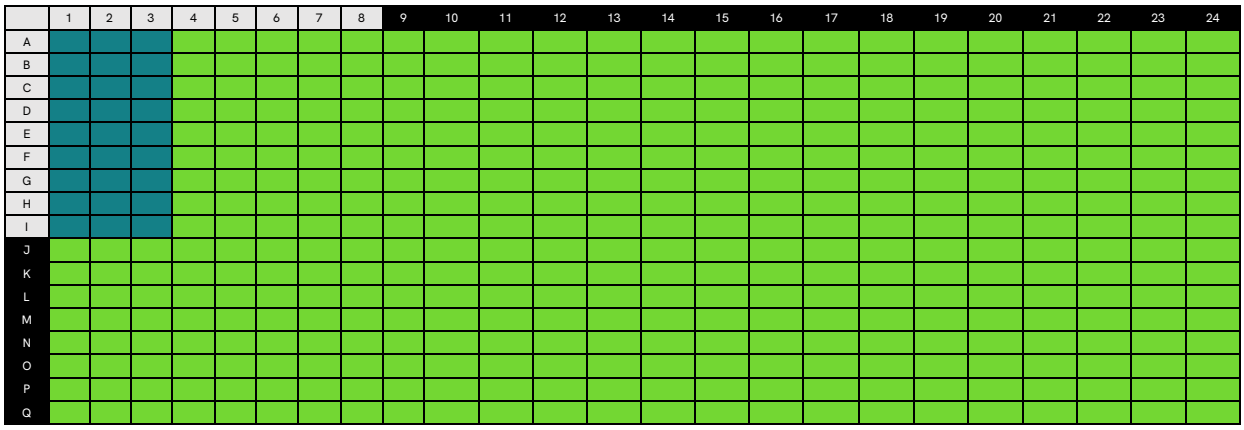
- Each well requires 5 µL of sample.
- Just after their collection, put the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at -60°C or below. Avoid multiple freeze/thaw cycles.
- Samples with a concentration above the highest standard (Std 7) must be diluted diluent #5 (1X) or in your appropriate sample medium.
- In order to measure AAV5 in cell lysates, cells must be lysed with Lysis Buffer #3 (1X) for 30 min at RT under gentle shaking.

ASSAY PROTOCOL

		STANDARD (STD 0 – STD 7)	SAMPLES
Step 1		Dispense 5 µL of each AAV5 standard (Std 0 - Std 7) into each standard well. And add 5 µL of diluent #5 (1X)	Dispense 5 µL of each sample into each sample well. And add 5 µL of diluent #5 (1X)
Step 2		Add 5 µL of AAV5 d2 antibody working solution to all wells	
Step 3		Add 5 µL of Premix with donor working solution to all wells.	
Step 4		Seal the plate and incubate Overnight at RT Following incubation, the signal remains stable over a period of 48 hours.	
Step 5		Remove the plate sealer and read on an HTRF® compatible reader	

	1	2	3	4	5	6
A	5 µL Std 0 (Negative control) 5 µL of diluent #5 5 µL AAV5 d2 5 µL Premix	Repeat Well A1	Repeat Well A1	5 µL sample 1 5 µL diluent #5 5 µL AAV5 d2 5 µL Premix	Repeat Well A4	Repeat Well A4
B	5 µL Std 1 5 µL of diluent #5 5 µL AAV5 d2 5 µL Premix	Repeat Well B1	Repeat Well B1	5 µL sample 2 5 µL diluent #5 5 µL AAV5 d2 5 µL Premix	Repeat Well B4	Repeat Well B4
C	5 µL Std 2 5 µL of diluent #5 5 µL AAV5 d2 5 µL Premix	Repeat Well C1	Repeat Well C1	5 µL sample 3 5 µL diluent #5 5 µL AAV5 d2 5 µL Premix	Repeat Well C4	Repeat Well C4
D	5 µL Std 3 5 µL of diluent #5 5 µL AAV5 d2 5 µL Premix	Repeat Well D1	Repeat Well D1	5 µL sample 4 5 µL diluent #5 5 µL AAV5 d2 5 µL Premix	Repeat Well D4	Repeat Well D4
E	5 µL Std 4 5 µL of diluent #5 5 µL AAV5 d2 5 µL Premix	Repeat Well E1	Repeat Well E1	5 µL sample 5 5 µL diluent #5 5 µL AAV5 d2 5 µL Premix	Repeat Well E4	Repeat Well E4
F	5 µL Std 5 5 µL of diluent #5 5 µL AAV5 d2 5 µL Premix	Repeat Well F1	Repeat Well F1	5 µL sample 6 5 µL diluent #5 5 µL AAV5 d2 5 µL Premix	Repeat Well F4	Repeat Well F4
G	5 µL Std 6 5 µL of diluent #5 5 µL AAV5 d2	Repeat Well G1	Repeat Well G1	5 µL sample ... 5 µL diluent #5 5 µL AAV5 d2 5 µL Premix	Repeat Well G4	Repeat Well G4

H	5 µL Premix 5 µL Std 7 5 µL of diluent #5 5 µL AAV5 d2 5 µL Premix	Repeat Well H1	Repeat Well H1		5 µL sample ... 5 µL diluent #5 5 µL AAV5 d2 5 µL Premix	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 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.

$$\text{delta Ratio} = \text{Ratio Standard or sample} - \text{Ratio Standard 0}$$

3) 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

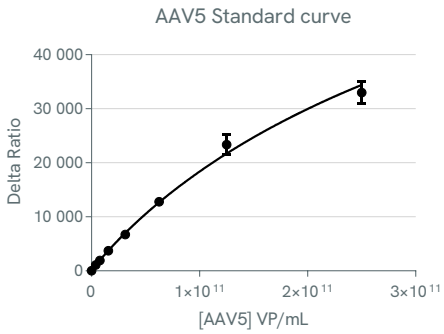
This data must not be substituted for the data obtained in the laboratory and should be considered only as an example.

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

Standard curve fitting with the 4 Parameter Logistic (4PL with $1/Y^2$) model

		Ratio (1)	Delta Ratio (2)	CV% (3)
Std 0	Negative control	1045	0	0%
Std 1	3.91E+09 VP/mL	2120	1075	2%
Std 2	7.81E+09 VP/mL	2975	1930	4%

Std 3	1.56E+10 VP/mL	4750	3705	3%
Std 4	3.13E+10 VP/mL	7749	6704	5%
Std 5	6.25E+10 VP/mL	13831	12786	5%
Std 6	1.25E+11 VP/mL	24389	23344	8%
Std 7	2.50E+11 VP/mL	34030	32985	6%



REACH European regulations and compliance