



LTB4 KITS

PROTOCOL

Part # 64LB4PEG & 64LB4PEH

Test size#: 500 tests (64LB4PEG) and 10,000 tests (64LB4PEH) - assay volume: 20 μ L

Revision: 05-july 2020

Store at: -60°C or below (64LB4PEG); -60°C or below (64LB4PEH)

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

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of Leukotriene B4 (LTB4) 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, LTB4 is detected in a competitive assay by using anti LTB4 antibody labeled with Europium cryptate (donor), and LTB4 labeled with XL665 (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 LTB4 present in the sample competes with the binding between the two HTRF detection solutions and thereby prevents FRET from occurring. The specific signal is inversely proportional to the LTB4 concentration.

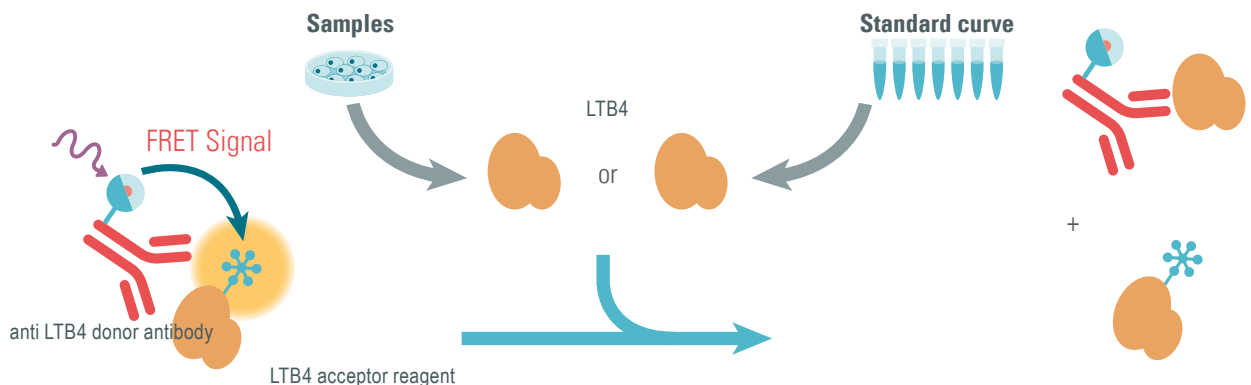
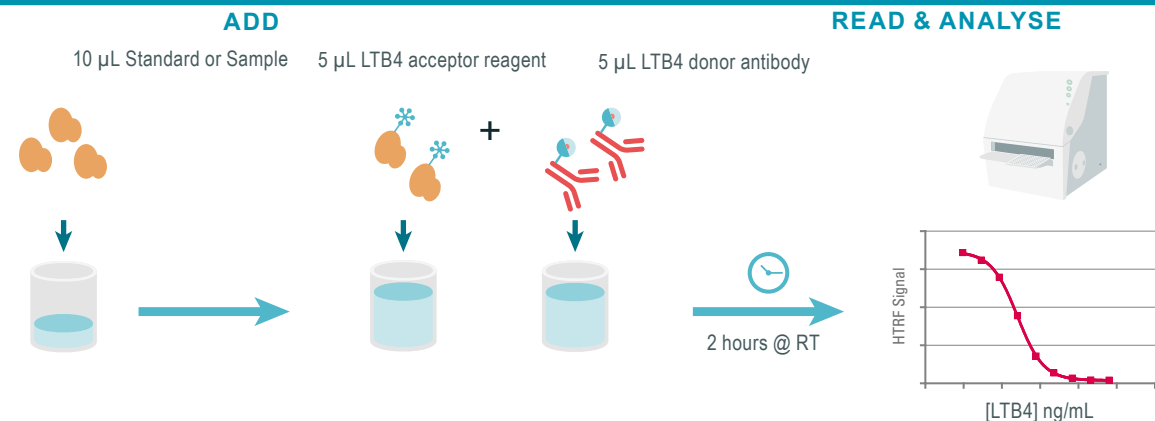


Figure 1: Principle of HTRF LTB4 competitive assay.

PROTOCOL AT A GLANCE



Do not pre-mix the XL665 and Cryptate solutions prior to dispensing.

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

MATERIALS PROVIDED:

KIT COMPONENTS	500 TESTS * CAT # 64LB4PEG	10,000 TESTS * CAT # 64LB4PEH
LTB4 Standard Frozen	1 vial - 50 µL 10 µg/mL - Ethanol	1 vial - 50 µL 10 µg/mL - Ethanol
anti LTB4 antibody Eu Cryptate antibody	1 vial - 60 µL Frozen - 50X	1 vial - 1.2 mL Frozen - 50X
LTB4 XL665 reagent	1 vial - 50 µL Frozen - 50X	1 vial - 1 mL Frozen - 50X
Diluent ** ready-to-use	1 vial 20 mL	1 vial 20 mL
Detection buffer *** 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 cisbio.com/compatible-readers

- Small volume (SV) detection microplates - .

For more information about microplate recommendations, please visit our website at: cisbio.com/microplates-recommendations

STORAGE AND STABILITY

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






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 .

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.
- Thaw all reagents at room temperature, allow them to warm up.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- LTB4 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.

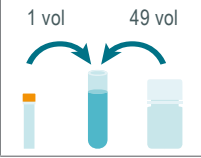



TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 64LB4PEG			10,000 TESTS KIT - 64LB4PEH
anti LTB4 antibody Eu Cryptate antibody			
Thaw the anti LTB4 antibody Eu Cryptate antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below.			Thaw the anti LTB4 antibody Eu Cryptate antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below.
LTB4 XL665 reagent			
Thaw the LTB4 XL665 reagent . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below.			Thaw the LTB4 XL665 reagent . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below.
LTB4 Standard			
Thaw the LTB4 standard solution in order to obtain a 10 µg/mL (see vial label) stock solution. Mix gently.			Thaw the LTB4 standard solution in order to obtain a 10 µg/mL (see vial label) stock solution. Mix gently.
Diluent			
The diluent is ready-to-use			The diluent is ready-to-use
Detection buffer			
The Detection buffer is ready-to-use.			The Detection buffer is ready-to-use.w

TO PREPARE ANTIBODY WORKING SOLUTIONS:

Each well requires 5 µL anti LTB4 antibody Eu Cryptate antibody and 5 µL LTB4 XL665 reagent.

Prepare the two solutions in separate vials.

500 TESTS KIT - 64LB4PEG			10,000 TESTS KIT - 64LB4PEH
anti LTB4 antibody Eu Cryptate antibody			
Dilute 50-fold the stock solution of LTB4 Eu cryptate antibody with detection buffer#4 e.g. take 0.05 mL of Eu Cryptate antibody stock solution and add it to 2.45 mL of detection buffer #4. Filter the Cryptate antibody working solution before use (on Millex GV 0.22µm as example). A loss of 15% of the volume can be expected.			Dilute 50-fold the stock solution of LTB4 Eu Cryptate antibody with detection buffer#4 e.g. take 1 mL of Eu Cryptate antibody stock solution and add it to 49 mL of detection buffer #4. Filter the Cryptate antibody working solution before use (on Millex GV 0.22µm as example). A loss of 15% of the volume can be expected.
LTB4 XL665 reagent			
Dilute 50-fold the stock solution of LTB4 XL665 reagent with detection buffer#4: e.g. take 0.05 mL of XL665 reagent stock solution and add it to 2.45 mL of detection buffer #4.			Dilute 50-fold the stock solution of LTB4 XL665 reagent with detection buffer#4: e.g. take 1 mL of XL665 reagent stock solution and add it to 49 mL of detection buffer #4.
Antibody mix			
Do not pre-mix the XL665 and the Eu Cryptate solutions prior to dispensing.			

TO PREPARE STANDARD WORKING SOLUTIONS:

- Each well requires 10 μL of standard.
- Dilute the standard stock solution serially with diluent
- Due to the sticky property of LTB4 on albumin, do not use buffers containing albumin. We recommend preparing standard curve in the diluent provided in the kit (casein 0.1%). Buffer containing fetal calf serum up to 2% can be used to replace the diluent. Due to its instability, the LTB4 standard curve must be prepared just before use (avoid repeated freezing and thawing). Centrifuge the vial of standard stock solution (green cap) before opening the cap. See concentration on the label.
- 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 .
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

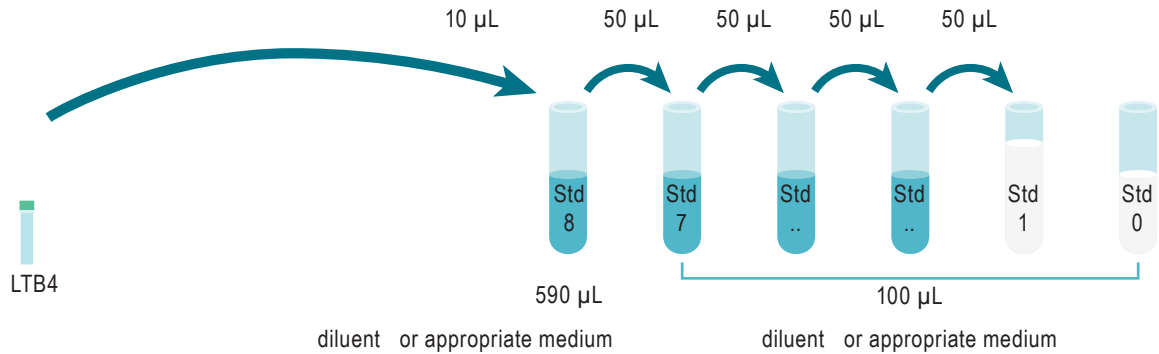
A recommended standard dilution procedure is listed and illustrated below:

Dilute the standard stock solution 60-fold with diluent to prepare high standard (Std 8): e.g. take 10 μL of standard stock solution and add it to 590 μL of diluent . Mix gently.

Use the high standard (Std 8) to prepare the standard curve using 1/3 serial dilutions as follows:

- Dispense 100 μL of diluent in each vial from Std 7 to Std 0.
- Add 50 μL of standard to 100 μL of diluent , mix gently and repeat the 1/3 serial dilution to make standard solutions: std7, std6, std5, std4, std3, std2, std1.

This will create 8 standards for the analyte. Std 0 (Positive control) is diluent or appropriate culture medium alone.








STANDARD	SERIAL DILUTIONS	LTB4 WORKING SOLUTION (ng/mL)
Standard Stock solution	Thawed stock solution	10,000
Standard 8	10 μL standard Stock solution + 590 μL Diluent	166.7
Standard 7	50 μL standard 8 + 100 μL Diluent	55.6
Standard 6	50 μL standard 7 + 100 μL Diluent	18.5
Standard 5	50 μL standard 6 + 100 μL Diluent	6.2
Standard 4	50 μL standard 5 + 100 μL Diluent	2.1
Standard 3	50 μL standard 4 + 100 μL Diluent	0.7
Standard 2	50 μL standard 3 + 100 μL Diluent	0.23
Standard 1	50 μL standard 2 + 100 μL Diluent	0.08
Standard 0	100 μL Diluent	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.
- Samples with a concentration above the highest standard (Std 8) must be diluted diluent or in your appropriate sample medium.

ASSAY PROTOCOL

		Negative control or Cryptate control	Standard (Std 0 - Std 8)	Samples
Step 1 		Dispense 10 μL of diluent into each negative control well	Dispense 10 μL of each LTB4 standard (Std 0 - Std 8) into each standard well	Dispense 10 μL of each sample into each sample well
Step 2 		Add 5 μL of detection buffer to all negative control wells	Add 5 μL LTB4 acceptor reagent working solution to all wells	
Step 3 		Add 5 μL LTB4 donor antibody working solution to all wells		
Step 4 		Seal the plate and incubate 2 hours @ RT		
Step 5 		Remove the plate sealer and read on an HTRF® compatible reader		

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.

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

3. Calculate the % delta F which reflects the signal to background of the assay. The negative control plays the role of an internal assay control. Delta F is used for the comparison of day to day runs of the same assay.

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

For more information about data reduction, please visit <http://www.cisbio.com/htrf-ratio-and-data-reduction>

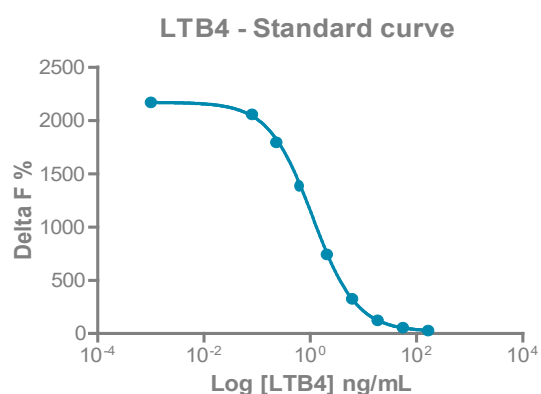
RESULTS

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 assay standard curve is created by plotting delta F% versus the analyte concentration.

	Ratio ⁽¹⁾	CV ⁽²⁾	Delta F% ⁽³⁾
Negative control	459	3.8%	
Std 0 – Positive control	8,923	0.3%	2,170%
Std 1 - 0.08 ng/mL	8,480	4.2%	2,057%
Std 2 - 0.23 ng/mL	7,444	0.2%	1,794%
Std 3 - 0.69 ng/mL	5,843	1.8%	1,386%
Std 4 - 2.06 ng/mL	3,315	5.2%	743%
Std 5 – 6.17 ng/mL	1,671	2.5%	325%
Std 6 - 18.52 ng/mL	885	2.4%	125%
Std 7 - 55.56 ng/mL	614	2.2%	56%
Std 8 - 166.67 ng/mL	507	1.4%	29%



ANALYTICAL CHARACTERISTICS

CROSS-REACTIVITY

	Cross-reactivity %
Leukotriene B4	100.00%
6-trans 12 epi LTB4	0.19%
6-trans LTB4	5.50%

DETECTION LIMIT

Detection limit - working concentration
1.12 ng/mL - 3.3 nM

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. Remaining disclaimer.

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