LTC4 KITS

PROTOCOL

Part # 64LC4PEG & 64LC4PEH

Test size#: 500 tests (64LC4PEG) and 10,000 tests (64LC4PEH) - assay volume: 20 µL

Revision: 04-May 2020

Store at: -60°C or below (64LC4PEG); -60°C or below (64LC4PEH)

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

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of Leukotriene C4 (LTC4) 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, LTC4 is detected in a competitive assay by using anti LTC4 antibody labeled with Europium cryptate (donor), and LTC4 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 LTC4 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 LTC4 concentration.

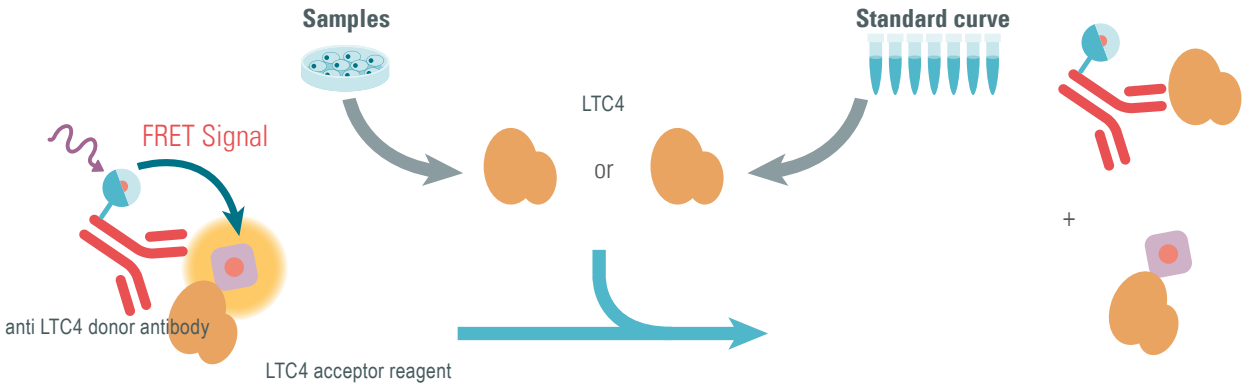
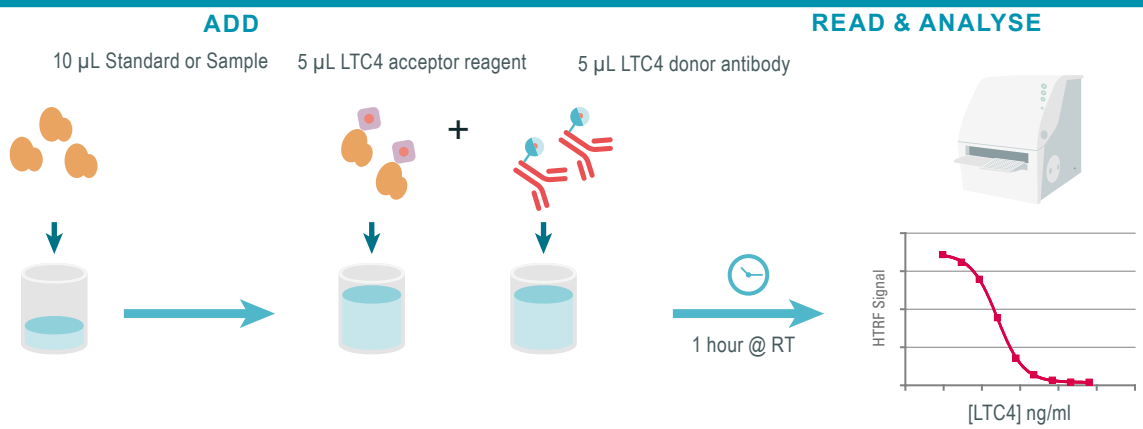


Figure 1: Principle of HTRF LTC4 competitive assay.

PROTOCOL 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.

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MATERIALS PROVIDED:

KIT COMPONENTS	500 TESTS * CAT # 64LC4PEG	10,000 TESTS * CAT # 64LC4PEH
LTC4 Standard	1 vial - 20 µL 100 µg/mL - Ethanol	1 vial - 20 µL 100 µg/mL - Ethanol
anti LTC4 antibody Eu Cryptate antibody	1 vial - 50 µL Frozen - 50X	1 vial - 1 mL Frozen - 50X
LTC4 d2 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

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.
- LTC4 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.

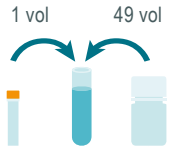
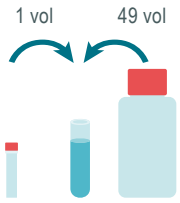
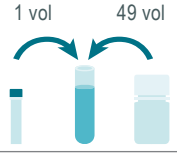
TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 64LC4PEG		10,000 TESTS KIT - 64LC4PEH	
anti LTC4 antibody Eu Cryptate antibody			
Thaw the anti LTC4 antibody Eu Cryptate antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below.			Thaw the anti LTC4 antibody Eu Cryptate antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below.
LTC4 d2 reagent			
Thaw the LTC4 d2 reagent . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below.			Thaw the LTC4 d2 reagent . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below.
LTC4 Standard			
Thaw the LTC4 standard solution in order to obtain a 100 µg/mL (see vial label) stock solution. Mix gently.			Thaw the LTC4 standard solution in order to obtain a 100 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 LTC4 antibody Eu Cryptate antibody and 5 µL LTC4 d2 reagent.

Prepare the two solutions in separate vials.

500 TESTS KIT - 64LC4PEG		10,000 TESTS KIT - 64LC4PEH	
anti LTC4 antibody Eu Cryptate antibody			
Dilute 50-fold the stock solution of LTC4 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.			Dilute 50-fold the stock solution of LTC4 Eu Cryptate antibody with detection buffer#3 e.g. take 1 mL of Cryptate antibody stock solution and add it to 49 mL of detection buffer #3.
LTC4 d2 reagent			
Dilute 50-fold the stock solution of LTC4 d2 reagent with detection buffer: e.g. take 0.05 mL of d2 reagent stock solution and add it to 2.45 mL of detection buffer #3.			Dilute 50-fold the stock solution of LTC4 d2 reagent with detection buffer: e.g. take 1 mL of d2 reagent stock solution and add it to 49 mL of detection buffer #3.
Antibody mix			
Do not pre-mix the d2 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 or in the medium used for the preparation of the samples
- Due to the sticky property of LTC4 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 LTC4 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.
- 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.

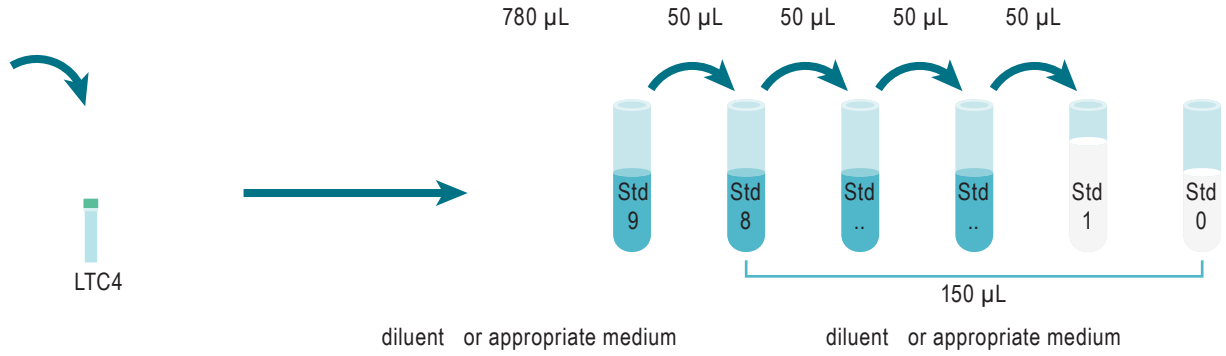
A recommended standard dilution procedure is listed and illustrated below:

Dilute the standard stock solution 40-fold with diluent to prepare high standard (Std 9): e.g. take 780 µL of standard stock solution and add it to of diluent . Mix gently.

Use the high standard (Std 9) to prepare the standard curve using 1/4 serial dilutions as follows:

- Dispense 150 µL of diluent in each vial from Std 8 to Std 0.
- Add 50 µL of standard to 150 µL of diluent , mix gently and repeat the 1/4 serial dilution to make standard solutions: std8, std7, std6, std5, std4, std3, std2, std1.

This will create 9 standards for the analyte. Std 0 (Positive control) is diluent or appropriate culture medium alone.



STANDARD	SERIAL DILUTIONS	LTC4 WORKING SOLUTION (ng/mL)
Standard Stock solution	Thawed stock solution	100,000
Standard 9	Add directly 780µL of diluent to the 20µL of standard stock solution	2,500
Standard 8	50 µL Std 9 + 150 µL Diluent	625
Standard 7	50 µL Std 8 + 150 µL Diluent	156.25
Standard 6	50 µL Std 7 + 150 µL Diluent	39.06
Standard 5	50 µL Std 6 + 150 µL Diluent	9.77
Standard 4	50 µL Std 5 + 150 µL Diluent	2.44
Standard 3	50 µL Std 4 + 150 µL Diluent	0.61
Standard 2	50 µL Std 3 + 150 µL Diluent	0.15
Standard 1	50 µL Std 2 + 150 µL Diluent	0.04
Standard 0	150 µL Diluent	0

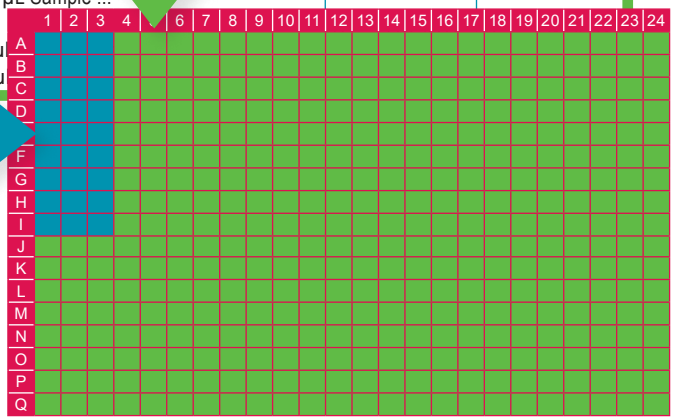
TO PREPARE SAMPLES:

- 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 9) must be diluted diluent or in your appropriate sample medium.

ASSAY PROTOCOL

	Negative control or Cryptate control	Standard (Std 0 - Std 9)	Samples
Step 1 	Dispense 10 µL of diluent into each negative control well	Dispense 10 µL of each LTC4 standard (Std 0 - Std 9) 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 LTC4 acceptor reagent working solution to all wells	
Step 3 	Add 5 µL LTC4 donor antibody working solution to all wells		
Step 4 	Seal the plate and incubate 1 hour @ RT		
Step 5	Remove the plate sealer and read on an HTRF® compatible reader		

	1	2	3	4	5	6
A	10 µL diluent (Negative control) 5 µL Detection Buffer# 3 5 µL LTC4 donor antibody	Repeat Well A1	Repeat Well A1	10 µL Sample 1 5 µL LTC4 acceptor reagent 5 µL LTC4 donor antibody	Repeat Well A4	Repeat Well A4
B	10 µL Std 0 (Positive control) 5 µL LTC4 acceptor reagent 5 µL LTC4 donor antibody	Repeat Well B1	Repeat Well B1	10 µL Sample 2 5 µL LTC4 acceptor reagent 5 µL LTC4 donor antibody	Repeat Well B4	Repeat Well B4
C	10 µL Std 1 5 µL LTC4 acceptor reagent 5 µL LTC4 donor antibody	Repeat Well C1	Repeat Well C1	10 µL Sample 3 5 µL LTC4 acceptor reagent 5 µL LTC4 donor antibody	Repeat Well C4	Repeat Well C4
D	10 µL Std 2 5 µL LTC4 acceptor reagent 5 µL LTC4 donor antibody	Repeat Well D1	Repeat Well D1	10 µL Sample ... 5 µL LTC4 acceptor reagent 5 µL LTC4 donor antibody	Repeat Well D4	Repeat Well D4
E	10 µLStd ... 5 µL LTC4 acceptor reagent 5 µL LTC4 donor antibody	Repeat Well E1	Repeat Well E1	10 µL Sample ... 5 µL LTC4 acceptor reagent 5 µL LTC4 donor antibody	Repeat Well E4	Repeat Well E4
F	10 µL Std ... 5 µL LTC4 acceptor reagent 5 µL LTC4 donor antibody	Repeat Well F1	Repeat Well F1	10 µL Sample ... 5 µL LTC4 acceptor reagent 5 µL LTC4 donor antibody	Repeat Well F4	Repeat Well F4
G	10 µL Std ... 5 µL LTC4 acceptor reagent 5 µL LTC4 donor antibody	Repeat Well G1	Repeat Well G1	10 µL Sample ... 5 µL LTC4 acceptor reagent 5 µL LTC4 don	Repeat Well G4	Repeat Well G4
H	10 µL Std ... 5 µL LTC4 acceptor reagent 5 µL LTC4 donor antibody	Repeat Well H1	Repeat Well H1	10 µL Sample ... 5 µ 5 µ		



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.

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

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

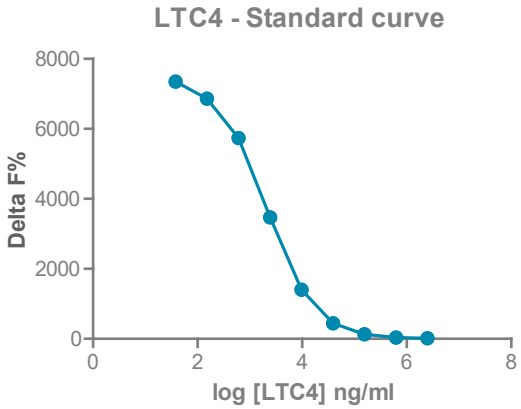
RESULTS

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	410	2.6%	
Std 0 – Positive control	30,758	0.4%	7,409%
Std 1 - 0.038 ng/mL	30,522	1%	7,351%
Std 2 - 0.153 ng/mL	28,513	1.2%	6,861%
Std 3 - 0.61 ng/mL	23,914	1.5%	5,738%
Std 4 - 2.44 ng/mL	14,626	3.5%	3,471%
Std 5 - 9.77 ng/mL	6,143	2.2%	1,400%
Std 6 - 39.06 ng/mL	2,214	2%	441%
Std 7 - 156.25 ng/mL	929	2.1%	127%
Std 8 - 625 ng/mL	571	0.9%	39%
Std 9 - 2500 ng/mL	467	2%	14%



ANALYTICAL CHARACTERISTICS

CROSS-REACTIVITY

	Cross-reactivity %
Leukotriene C4	100%
Leukotriene D4	100%
Leukotriene E4	100%
Leukotriene B4	<0.01%
Leukotriene A4	<2%

DETECTION LIMIT

Detection limit	< 0.1 ng/mL
EC50	2.7 ng/mL

FOR MORE INFORMATION
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Japan +81-(0)43-306-8712 Visit www.cisbio.com to find a list of our regional distributors

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