HUMAN FRATAXIN KITS

PROTOCOL

Part # 63ADK039PEG & 63ADK039PEH

Test Size#: 500 tests (63ADK039PEG), 10,000 tests (63ADK039PEH) - assay volume: 20 µL

Revision: 04-Nov.2020

Store at: ≤- 60°C (63ADK039PEG); ≤- 60°C (63ADK039PEH)

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

ASSAY PRINCIPLE

Cisbio Bioassays' Human Frataxin assay is only intended for quantitative measurement of Human Frataxin in cells using HTRF® technology.

Human Frataxin is detected in a sandwich assay format using 2 different specific antibodies, one labeled with Europium Cryptate (donor) and the second with d2 (acceptor).

The principle of detection 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). The two antibodies bind to the Human Frataxin present in the sample, thereby generating FRET. Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the Human Frataxin concentration (Fig. 1).

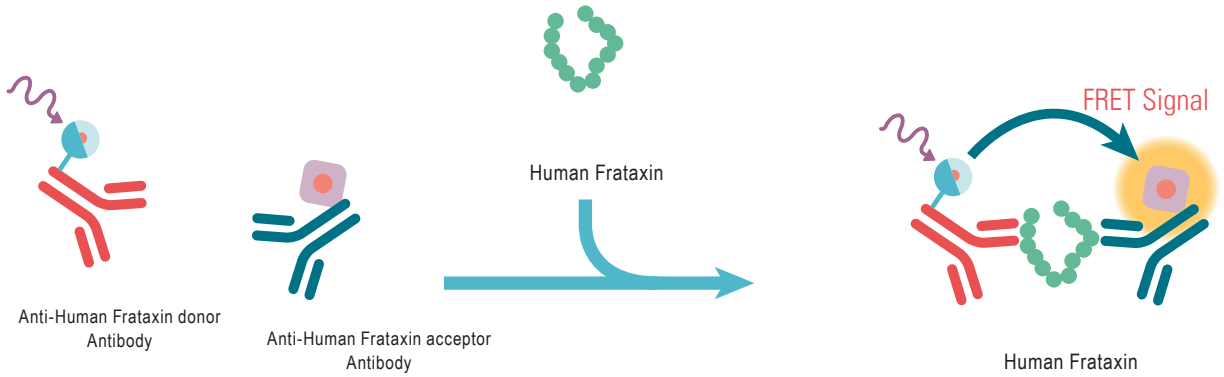
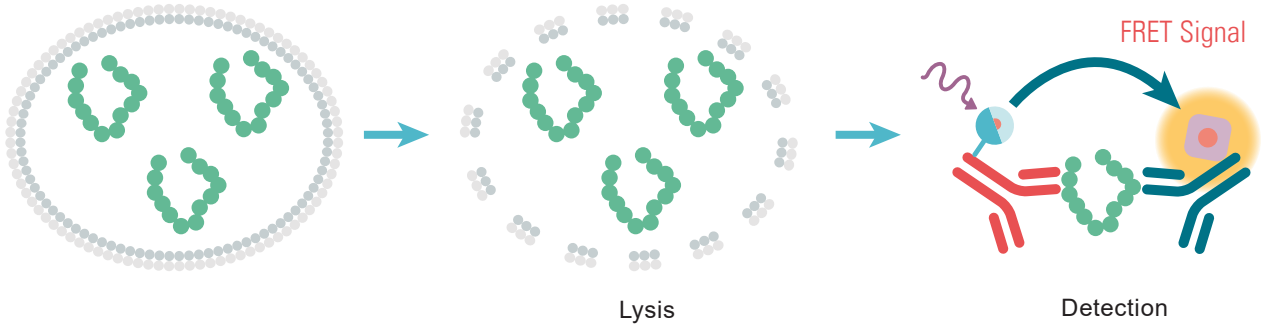


Figure 1: Principle of HTRF Human Frataxin sandwich assay.

The assay is run under a two-plate assay protocol, where cells are plated, stimulated and lysed in the same culture plate. Lysates are then transferred to the assay plate for the detection of Human Frataxin by HTRF® reagents. This protocol gives the cells viability and confluence to be monitored. It can also be further streamlined to a one-plate assay protocol. Detection of Human Frataxinwith HTRF® reagents is performed in a single plate used for plating, stimulation and detection. No washing steps are required. This protocol, HTS designed, allows miniaturization while maintaining HTRF® quality.

Technical support team can help you to set-up this protocol or another one.
Please contact us at www.cisbio.com/drug-discovery/contact-us

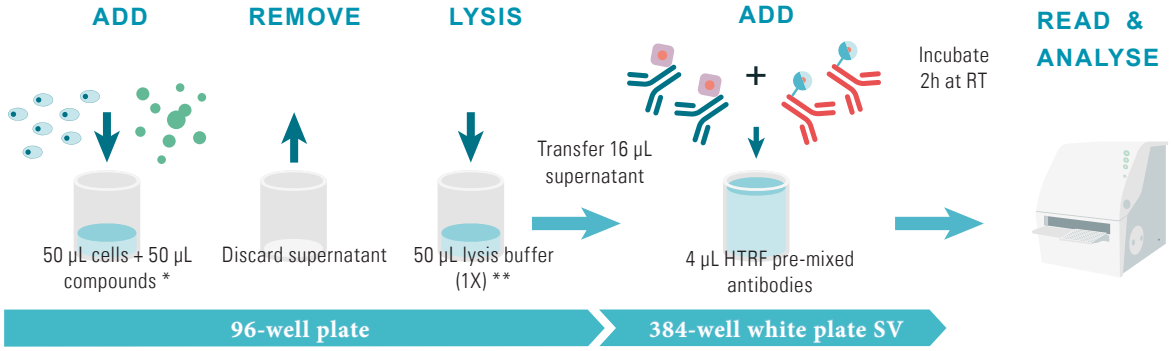
PROTOCOL AT A GLANCE



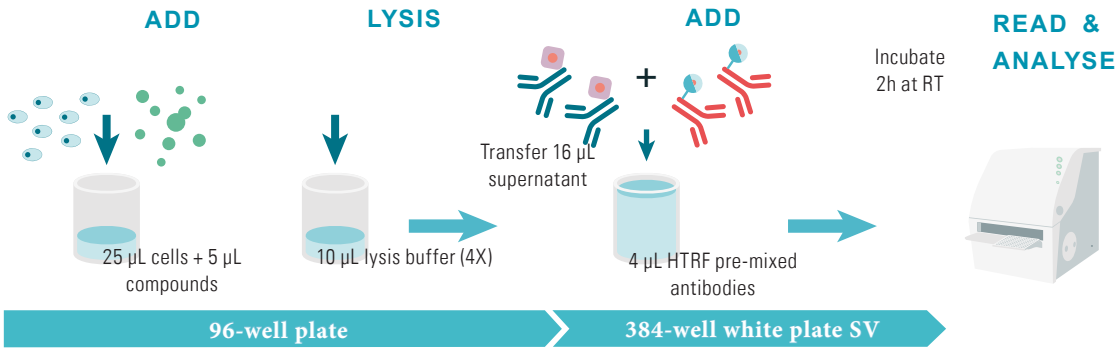
The assay can be run under a two-plate protocol, where cells are plated and stimulated in the same culture plate, then transferred to the assay plate for the HTRF® detection. This protocol enables the cells' viability and confluence to be monitored. It can also be further streamlined to a one-plate assay protocol where plating, stimulation and detection is performed in a single plate. Cell density, stimulation time, lysis step and other parameters related to the biology are cell-dependent and need to be optimized. For cells kept in medium for the lysis, we recommend to use the lysis buffer 4X (ready to use).

For cells removing the medium for lysis, we recommend to use the lysis buffer 1X.

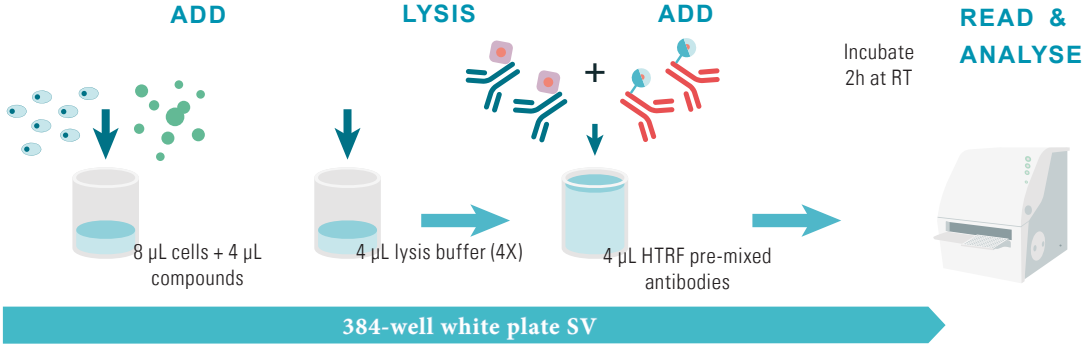
TWO-PLATE ASSAY PROTOCOL (FOR ADHERENT CELLS):



TWO-PLATE ASSAY PROTOCOL (FOR SUSPENSION CELLS):



ONE-PLATE ASSAY PROTOCOL:



MATERIALS PROVIDED:

Kit components	500 tests Cat # 63ADK039PEG	10,000 tests Cat # 63ADK039PEH
Control lysate Frozen/ready-to-use	1 vial - 150 µL	2 vials - 150µL
Anti-Human Frataxin-Eu Cryptate Antibody	1 vial - 20 µL Frozen - 50 X	1 vial - 400µL Frozen - 50 X
Anti-Human Frataxin-d2 Antibody	1 vial - 20 µL Frozen - 50 X	1 vial - 400 µL Frozen - 50 X
Lysis buffer #2 * stock solution 4X	1 vial - 16 mL Frozen	1 vial - 70 mL Frozen
Detection Buffer #8 ** ready-to-use	1 vial - 2 mL Frozen	1 vial - 50 mL Frozen

* Amounts of reagents provided are sufficient for generating 50 µL of cell lysate per well.
** 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 www.cisbio.com/compatible-readers
- Small volume (SV) detection microplates - Use white plate only.
- For more information about microplate recommendations, please visit our website at: cisbio.com/microplates-recommendations

STORAGE AND STABILITY

Antibodies, control lysate and buffers should be stored frozen until use.
Thawed lysis buffer and detection buffer can be stored at 2-8°C in your premises. Thawed antibodies are stable 48 hours at 2-8°C; they can be refrozen (at ≤-16°C) and thawed at least one more time.
Control lysate must be stored frozen at -60°C or below. Thawed control lysate can be refrozen (at -60°C) and thawed one more time.

REAGENT PREPARATION

Allow all reagents to thaw before use. We recommend centrifuging the vials gently after thawing, before pipeting the stock solutions.

Prepare the working solutions from stock solutions by following the instructions below.

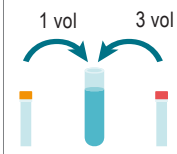
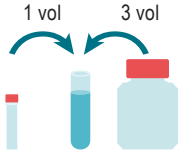
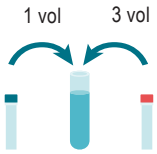
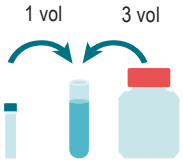
POSITIVE CONTROL SOLUTION: READY-TO-USE

The control cell lysate is only provided as an internal assay control to check the quality of the results obtained. The window between control lysate and negative control should be greater than 2.

TO PREPARE WORKING ANTIBODY SOLUTIONS:

HTRF® reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of the d2 and Cryptate-antibodies will impair the assay's quality. Be careful, as working solution preparation for antibodies may differ between the 500 and 10,000 tests data point kit.

Antibody working solutions are stable for 2 days at 4°C. Dilute the antibodies with detection buffer #8.

500 TESTS KIT - 63ADK039PEG		10,000 TESTS KIT - 63ADK039PEH	
Anti-Human Frataxin- Cryptate antibody			
Dilute 50-fold the frozen stock solution with detection buffer#8: e.g. add 980 µL of detection buffer to the 20 µL of Cryptate-antibody stock solution.			Dilute 50-fold the frozen stock solution with detection buffer#8: e.g. add 19.6 mL of detection buffer to the 0.4 mL of Cryptate-antibody stock solution.
Anti-Human Frataxin-d2 antibody			
Dilute 50-fold the frozen stock solution with detection buffer: e.g. add 980 µL of detection buffer to the 20 µL of d2-antibody stock solution.			Dilute 50-fold the frozen stock solution with detection buffer: e.g. add 19.6 mL of detection buffer to the 0.4 mL of d2-antibody stock solution.
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.			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.

TO PREPARE LYSIS BUFFER:

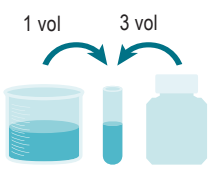
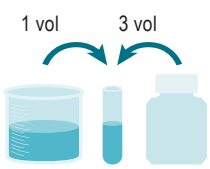
Make sure that the lysate has been generated by using the kit reagents.

Prepare the required amount of lysis buffer before running the assay, working solutions are stable for 2 days at 2-8°C.

➤ Lysis buffer 1X for two-plate assay protocol on adherent cells

Determine the amount of lysis buffer needed for the experiment. Each well requires generally 50 µL of lysis buffer.

Prepare a lysis buffer solution 1X by diluting 4-fold the lysis buffer 4X with distilled water.

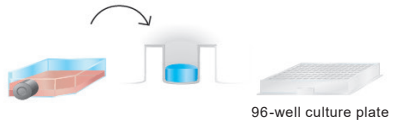
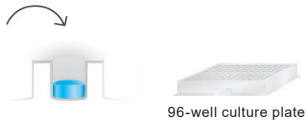
500 TESTS KIT - 63ADK039PEG		10,000 TESTS KIT - 63ADK039PEH	
Preparation of lysis buffer 1X			
Dilute the "lysis buffer 4X" 4-fold with distilled water to prepare lysis buffer 1X. e.g. take 1.25 mL of lysis buffer 4X and add it to 3.75 mL of distilled water. Mix gently.			Dilute the "lysis buffer 4X" 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.

➤ Lysis buffer 4X for assay protocol on cells: two-plate assay protocol on suspension cells and one-plate assay protocol

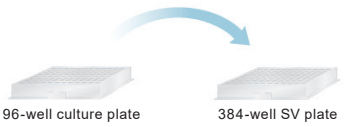
Buffer is ready-to-use
Determine the amount of lysis buffer needed for the experiment:
- One-plate assay protocol: Each well requires generally 4 µL of lysis buffer.
- Two-plate assay protocol: Each well requires generally 10 µL of lysis buffer.

TWO PLATE ASSAY PROTOCOL

GENERAL LAB WORK PRIOR USING CISBIO KIT: CELLS PREPARATION

	FOR ADHERENT CELLS	FOR SUSPENSION CELLS	
1	Plate 50 µL of cells in 96-well tissue-culture treated plate in appropriate growth medium and incubate overnight, at 37°C in CO2 atmosphere.	Plate 25 µL of cells in 96-well tissue-culture treated plate in appropriate growth medium and incubate overnight, at 37°C in CO2 atmosphere.	 96-well culture plate
	Optimization of cell seeding densities is recommended. Depending on receptor a starving step with serum-free medium could be essential.		
2	Dispense 50 µL of compounds (2X) diluted in cell culture medium	Dispense 5 µL of compounds (6X) diluted in cell culture medium	 96-well culture plate
	For most compound, incubation time should be above 24 hours at 37°C. We recommend a time course study to determine the optimal stimulation time. Note that concentration above 0.5% DMSO will impair assay performances. Same final concentration of DMSO must be used for each compound dilutions.		
3	Remove carefully cell supernatant either by aspirating supernatant or by flicking the plate.	Do not remove cell supernatant	 96-well culture plate

HUMAN FRATAXIN DETECTION USING CISBIO KIT

	FOR ADHERENT CELLS	FOR SUSPENSION CELLS	
4	Immediately add 50 µL of lysis buffer (1X) and incubate for at least 30 minutes at room temperature under shaking.	Immediately add 10 µL of lysis buffer (4X) and incubate for at least 30 minutes at room temperature under shaking.	 96-well culture plate
	Use the appropriate lysis buffer and incubate at room temperature with shaking. We recommend a time course study to determine the optimal lysis incubation time.		
5	After homogenization by pipeting up and down, transfer 16 µL of cell lysate from the 96-well cell-culture plate to a 384-well small volume white plate. Depending on cell lines used, it can be necessary to dilute the cell lysate to ensure samples are within the assay linear range		 96-well culture plate 384-well SV plate
6	Add 4 µL of premixed antibody solutions (vol/vol) prepared in the detection buffer #8. Cover the plate with a plate sealer. Incubate 2h at RT. Maximum signal is reached after 2h incubation time, and remains stable over a period of 24 hours. Therefore, readings can be made between 2h and 24h of incubation time. Set up your reader for Eu Cryptate and read the fluorescence emission at two different wavelenghts (665nm and 620nm) on a compatible HTRF® reader*.		 384-well SV plate

* For more information about HTRF® compatible readers and for set-up recommendations, please visit our website at: www.cisbio.com/drug-discovery/htrf-compatible-readers

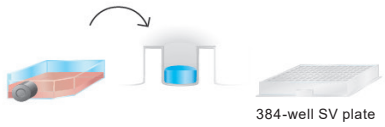
➤ Standard protocol for two-plate assay protocol in 20 µL final volume (after lysis step)

	Non treated cell lysate	Treated cell lysate	Positive control	Negative control	Blank control
Step 1	Dispense 16 µL of non treated cell lysate	Dispense 16 µL of treated cell lysate	Dispense 16 µL of control lysate	Dispense 16 µL of lysis buffer 1X	Dispense 16 µL of non treated cell lysate
Step 2	Add 2 µL of Anti-Human Frataxin-d2 working solution to all wells				Add 2 µL of detection buffer #8
Step 3	Add 2 µL of Anti Human Frataxin-Eu Cryptate working solution to all wells				
Step 4	Cover the plate with a plate sealer. Incubate 2h at room temperature. Maximum signal is reached after 2h incubation time, and remains stable over a period of 24 hours. Therefore, readings can be made between 2h and 24h of incubation time.				
Step 5	Remove the plate sealer and read on an HTRF® compatible reader				

The blank control is used to check the Cryptate signal at 620 nm.
The Negative control is used to check the non-specific signal. The ratio between control lysate signal / non-specific signal should be greater than 2.

ONE PLATE ASSAY PROTOCOL

GENERAL LAB WORK PRIOR USING CISBIO KIT: CELLS PREPARATION

1	Plate 8 µL of cells in a 384-well small volume white plate in your appropriate medium (without phenol red). Optimization of cell seeding densities is recommended. Depending on receptor a starving step with serum-free medium can be included.	
2	Dispense 4 µL of compounds (3X) diluted in your appropriate medium. For most compound, incubation time is between 10 and 30 minutes at 37°C. We recommend a time course study to determine the optimal stimulation time.	

HUMAN FRATAXIN DETECTION USING CISBIO KIT

3	Add 4 µL of lysis buffer (4X). Use the appropriate supplemented lysis buffer and incubate for at least 30 minutes at room temperature under shaking. We recommend a time course study to determine the optimal lysis incubation time.	
4	Add 4 µL of premixed antibody solutions (vol/vol) prepared in the detection buffer #8. Cover the plate with a plate sealer. Incubate 2h at RT at room temperature. Maximum signal is reached after 2h incubation time, and remains stable over a period of 24 hours. Therefore, readings can be made between 2h and 24h of incubation time. Maximum signal is reached after 2h incubation time, and remains stable over a period of 24 hours. Therefore, readings can be made between 2h and 24h of incubation time. Set up your reader for Eu Cryptate and read the fluorescence emission at two different wavelenghts (665nm and 620nm) on a compatible HTRF® reader*.	

* For more information about HTRF® compatible readers and for set-up recommendations, please visit our website at: www.cisbio.com/drug-discovery/htrf-compatible-readers

➤ **Standard protocol for one-plate assay protocol in 20 µL final volume**

		Non treated cell lysate	Treated cell lysate	Blank control	Negative control	Positive control
GENERAL LAB WORK	Step 1	Dispense 8 µL of cells			-	
	Step 2	Add 4 µL of your appropriate medium	Add 4 µL of compound	Add 4 µL of your appropriate medium	Dispense 12 µL of your appropriate medium	Dispense 16 µL of control lysate
HUMAN FRATAXIN DETECTION STEPS	Step 3	Add 4 µL of lysis buffer #2				-
	Step 4	Incubate for at least 30 minutes at room temperature under shaking				
	Step 5	Add 2 µL of Anti-Human Frataxin-d2 working solution to all wells		Add 2 µL of detection buffer #8	Add 2 µL of Anti-Human Frataxin-d2 working solution to all wells	
	Step 6	Add 2 µL of Anti Human Frataxin-Eu Cryptate working solution to all wells				
	Step 7	Cover the plate with a plate sealer. Incubate 2h at room temperature. Maximum signal is reached after 2h incubation time, and remains stable over a period of 24 hours. Therefore, readings can be made between 2h and 24h of incubation time.				
	Step 8	Remove the plate sealer and read on an HTRF® compatible reader				

The blank control is used to check the Cryptate signal at 620 nm.
The Negative control is used to check the non-specific signal. The ratio between control lysate signal / non-specific signal should be greater than 2.

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 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

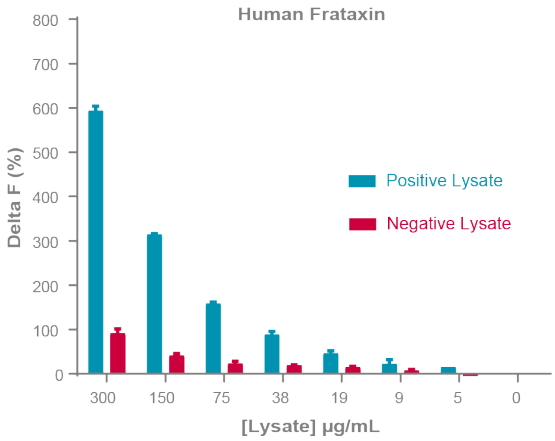
These data should be considered only as an example. Results may vary from one HTRF® compatible reader to another.

The curves are drawn up by plotting HTRF® Ratio versus the log [compound] concentrations.

Results were obtained on lymphoblasts

[lysate] µg/mL represents the concentration of the total protein in the assay

	Negative lysate			Positive lysate		
	Ratio (1)	CV% (2)	DF% (3)	Ratio (1)	CV% (2)	DF% (3)
0 (Neg. Control)	121	4%	-	125	7%	-
5 µg/mL	126	0%	4%	142	0%	13%
9 µg/mL	131	2%	9%	150	6%	20%
19 µg/mL	141	1%	17%	180	3%	44%
38 µg/mL	147	1%	21%	232	3%	85%
75 µg/mL	151	1%	25%	317	1%	154%
150 µg/mL	173	1%	43%	510	0%	307%
300 µg/mL	235	4%	94%	853	1%	582%



FOR MORE INFORMATION
Europe and other countries +33(0)466-796-705 U.S. and Canada 1-888-963-4567 China +86-21-5018-9880
Japan +81-(0)43-306-8712 Visit www.cisbio.com to find a list of our regional distributors

PerkinElmer® | cisbio