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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Interleukin-8 (human) TR-FRET Biomarker Assay Kit

Powered by Bioauxilium's THUNDER™ TR-FRET Technology

Item No. 500233

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item Name	96 wells Quantity/Size	480 wells Quantity/Size
400282	Europium-Labeled Interleukin-8 Antibody	1 vial/5 µl	1 vial/25 µl
400283	Acceptor-Labeled Interleukin-8 Antibody	1 vial/20 µl	1 vial/100 µl
400275	Assay Buffer 1 (5X)	1 vial/1 ml	3 vials/1 ml

To request this kit in bulk quantities, please contact our Sales department. If any of the items listed above are damaged or missing, please contact our Customer Service department. Sales and Customer Service departments can be reached at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

Do not mix or substitute reagents or materials from other kit lots or kits. Kits are quality control tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

We cannot guarantee the performance of the product outside the conditions detailed in this kit booklet.

Before You Start

Please note the following:

ONLY white plates should be used for TR-FRET.

DO NOT modify the assay protocol or volumes.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed at -80°C and used before the expiration date indicated on the outside of the box.

THUNDER™ General Information

THUNDER™ TR-FRET Biomarker Assay Kits are designed for the quantitative measurement of human cytokines in cell culture supernatants using homogeneous (no wash) TR-FRET technology.

THUNDER™ TR-FRET Biomarker Assay Kits are based on Bioauxilium's enhanced proprietary time-resolved Förster resonance energy transfer (TR-FRET) technology. THUNDER™ assays can be read on most commercially available TR-FRET-compatible plate readers (a list of suitable TR-FRET readers can be found at www.Bioauxilium.com). TR-FRET-based assays are homogeneous because they do not require any washing or separation steps. In addition, the THUNDER™ assays use a standardized, simple, and rapid "add-incubate-measure" protocol with a single step reagent addition. This streamlined assay protocol dramatically decreases hands-on time and provides a powerful alternative to cumbersome, error-prone and time-consuming techniques such as Western blot and ELISA.

THUNDER™ TR-FRET Biomarker Assay Kits do not require the addition of potassium fluoride to ensure signal stability. The non-toxic nature of THUNDER™ kits make them ideal candidates for multiplexing with other assay technologies using a sequential assay protocol.

THUNDER™ TR-FRET Biomarker Assay Kits contain the essential reagents necessary to carry out the measurement of human cytokines in cell culture supernatants, with the exception of the standard and the plate(s).

Materials Needed But Not Supplied

1. A plate reader equipped with a TR-FRET option
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. Low-volume 384-well, or half-area 96-well, white plates
5. Recombinant human IL-8 standard, available from ProSpec (Catalog No. CHM-327).
6. Adhesive sealing film for plates

INTRODUCTION

Background

Interleukin-8 (IL-8), also known as C-X-C motif chemokine ligand 8 (CXCL8), is a pro-inflammatory chemokine with roles in polymorphonuclear leukocyte infiltration and NETosis, as well as tumor cell proliferation, migration, and metastasis.[1-3] It is secreted by various cell types, including blood monocytes, alveolar macrophages, endothelial cells, epithelial cells, and fibroblasts, in response to inflammatory cytokines or environmental stressors such as hypoxia, reactive oxygen species (ROS), or bacterial particles.[2,3] IL-8 signals through two G protein-coupled receptors, chemokine C-X-C motif receptor 1 (CXCR1) and CXCR2, to induce neutrophil recruitment and NETosis at sites of infection.[3] Production of IL-8 is dysregulated in various inflammatory diseases, including chronic obstructive pulmonary disease (COPD), asthma, cystic fibrosis, and ulcerative colitis, as well as various cancers.[2,3]

About This Assay

This IL-8 (human) TR-FRET Biomarker Assay Kit uses a homogenous TR-FRET sandwich immunoassay method amenable to rapid measurement of IL-8 levels in cell culture supernatants. The standard curve spans a dynamic range of 15-10,000 pg/ml and a lower limit of quantification (LLOQ) of 74 pg/ml using the assay buffer included in the kit with laser excitation. The amount of reagents provided is sufficient for testing either 96 or 480 wells, depending on the size of the kit.

Principle Of This Assay

This assay is a homogenous TR-FRET-based sandwich immunoassay (Figure 1, below). Native or recombinant human IL-8 in cell culture supernatant samples is detected with a pair of fluorophore-labeled antibodies reactive to human IL-8.

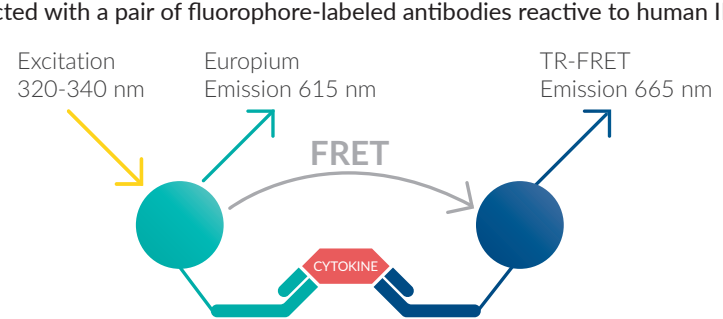

Figure 1. Schematic of the TR-FRET biomarker assay principle

The first antibody is labeled with a long-lifetime donor fluorophore (a europium chelate; Eu-Ab) and the second with a far-red acceptor fluorophore (FR-Ab). The binding of the two labeled antibodies to distinct epitopes on the target cytokine takes place in solution and brings the two dyes into close proximity. Excitation of the donor Eu chelate molecules with a flash lamp (320 or 340 nm) or laser (337 nm) triggers a FRET from the donor to the acceptor molecules, which, in turn, emit a TR-FRET signal at 665 nm. The signal at 665 nm is proportional to the concentration of human IL-8 in the cell culture supernatant. Residual energy from the Eu chelate generates light at 615 nm.

TR-FRET assays exhibit very low background fluorescence levels and high signal-to-background (S/B) ratios. The data can be expressed and analyzed as either the signal at 665 nm or the 665 nm/615 nm ratio. The ratiometric measurement further increases assay reproducibility and robustness.

PRE-ASSAY PREPARATION

Reagent Preparation

The instructions described below are for testing the entire number of wells in each kit. Adjust volumes accordingly when testing fewer wells. Prepare only as much reagent as is needed on the day of the assay.

Bring all reagents to room temperature prior to use.

Centrifuge all tubes before use to improve recovery of content (2,000 x g, 10-15 seconds).

Use ultrapure water (18 MΩ·cm) to dilute the Assay Buffer 1 (5X).

NOTE: It is recommended to test all standards in triplicate and samples at least in duplicate.

1. **Assay Buffer 1 (1X)**

   Mix the Assay Buffer 1 (5X) (Item No. 400275) end-over-end before use. The thawed Assay Buffer 1 (5X) will be stable for 1-2 weeks when stored at 4°C or for longer periods of time when stored at -80°C.

   Add 1 ml (96 wells) or 3 ml (480 wells) of Assay Buffer 1 (5X) with 4 ml (96 wells) or 12 ml (480 wells) of ultrapure water. Store unused Assay Buffer 1 (1X) at 4°C; it will be stable for approximately two days.

2. IL-8 Standard Serial Dilutions

Recombinant human IL-8 is not included in the kit. The kit has been validated using recombinant human IL-8 from ProSpec (Catalog No. CHM-327). Other commercial recombinant human IL-8 proteins may also work but have not been tested with the kit.

Prepare standard solutions just before use and use within one hour. Do NOT store the standard solutions.

Each well requires 15 µl of serially diluted standards.

Reconstitute the analyte following the manufacturer's instructions (typically 100-200 µg/ml) to create a stock solution. Dilute the stock solution in either Assay Buffer 1 (1X) or culture medium containing 10% FBS, depending on which will be used for the standard curve dilutions, to create a 1 µg/ml IL-8 working stock solution. The working stock solution will be used to prepare a standard dilution series.

NOTE: If all of the working stock solution will not be used within one hour, aliquot into screw-capped polypropylene vials and store at -80°C for long-term storage. The undiluted stock solution may also be stored at -80°C.

To prepare the standard solutions for use: obtain 11 clean test tubes and label them #1-11. Alternatively, serial dilutions can be conducted using a 96-well polypropylene plate.

Prepare serial dilutions for the standard curve as indicated in Table 1, on page 11, using either Assay Buffer 1 (1X) or culture medium containing 10% FBS. Change the pipette tip between each standard dilution. Mix each tube thoroughly between each transfer.

Tube	Volume of IL-8 Standard	Volume of Assay Buffer 1 (1X) OR Culture Medium + 10% FBS (µl)	IL-8 in standard curve (g/ml in 15 µl)
1	6 µl diluted IL-8 (1 µg/ml)	194	3.0E-8
2	30 µl of tube 1	60	1.0E-8
3	30 µl of tube 2	70	3.0E-9
4	30 µl of tube 3	60	1.0E-9
5	30 µl of tube 4	70	3.0E-10
6	30 µl of tube 5	60	1.0E-10
7	30 µl of tube 6	70	3.0E-11
8	30 µl of tube 7	60	1.0E-11
9	30 µl of tube 8	70	3.0E-12
10	30 µl of tube 9	60	1.0E-12
11*	0	70	0

Table 1. Standard curve serial dilutions
*In order to calculate the limit of detection (LOD), three additional zero-standard tubes per plate should be prepared and assayed in triplicate.

3. **Antibody Detection Mix (4X)**

NOTE: Due to the low reagent volumes, the antibodies are diluted with Assay Buffer 1 (1X) directly in the vial when assaying only 96 wells.

NOTE: Each well requires 5 µl of Antibody Detection Mix (4X).

Prepare and mix just before use.

Antibody Detection Mix (4X) (96 wells): Add 255 µl of Assay Buffer 1 (1X) into the vial containing 5 µl of Europium-Labeled IL-8 Antibody (Eu-Ab) (Item No. 400282). Add 240 µl of Assay Buffer 1 (1X) into the vial containing 20 µl of Acceptor-Labeled IL-8 Antibody (FR-Ab) (Item No. 400283). Gently mix the diluted Eu-Ab and FR-Ab solutions together.

OR

Antibody Detection Mix (4X) (480 wells): Gently mix 25 µl of Eu-Ab (Item No. 400282) with 1,275 µl of Assay Buffer 1 (1X). Gently mix 100 µl of FR-Ab (Item No. 400283) with 1,200 µl of Assay Buffer 1 (1X). Gently mix the diluted Eu-Ab and FR-Ab solutions together.

Store unused Antibody Detection Mix (4X) at 4°C; it will be stable for approximately two days. If all unused Antibody Detection Mix (4X) will not be used within two days, aliquot and store at -80°C. Avoid repeated freeze/thaw cycles.

Sample Preparation

Each well requires 15 µl of sample (cell culture supernatant).

After collection, place the samples at 4°C and assay immediately or aliquot and store at -80°C.

Avoid repeated freeze/thaw cycles. Thaw samples at room temperature. Do NOT use a water bath to thaw samples.

Prepare serial dilutions using the same media used to culture the cells. If the analyte concentration in the sample possibly exceeds the highest point of the standard curve, prepare one or more 10-fold dilutions of the sample.

ASSAY PROTOCOL

Workflow

The THUNDER™ TR-FRET Cell Signaling Assay workflow consists of a one-step assay reagent addition followed by an incubation period.

Figure 2. Assay workflow

Performing the Assay

Samples and standards MUST be assayed at least in duplicate (triplicate recommended) each time the assay is performed.

When loading reagents in the low-volume 384-well microplate, change pipette tips between each standard or sample addition and after each set of reagents.

If using a multichannel pipettor, always use a new disposable reagent reservior.

When reagents are added to the microplate, ensure the liquids are at the bottom of the well.

1. Add 15 µl of each working standard or 15 µl of the supernatant sample to the designated wells.
2. Add 5 µl of Antibody Detection Mix (4X) to each of the assay wells.
3. Cover the plate with adhesive plate-sealing film and incubate for 1 hour at room temperature.
4. Gently remove the adhesive plate-sealing film. Read the plate on a TR-FRET-compatible microplate reader (see Table 2, page 15).

NOTE: The same plate can be read several times without a negative effect on the assay performance.

TR-FRET Plate Reader Settings

We recommend reading the TR-FRET assays at two wavelengths, detecting both the emission from the Eu chelate donor fluorophore at 615 nm and the acceptor fluorophore at 665 nm. Table 2, below, provides instrument settings to be used as guidelines.

	TR-FRET-compatible Plate Reader	
Parameter	**Flash lamp excitation**	**Laser excitation**
Excitation filter	320 nm (or 340 nm)	N/A
Emission filter	615 nm (or 620 nm)	615 nm (or 620 nm)
Delay time	90 µs	50 µs
Flash energy level	100% or High	100%
Number of flashes	100-200	20
Window (integration time)	300 µs	100 µs

Table 2. Recommended TR-FRET plate reader settings

ANALYSIS

Calculations

Because the TR-FRET signal is read in a time-resolved manner, background subtraction is usually not necessary.

Do not use a value of zero for the zero standard. Use the actual background values obtained with the zero standard.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

1. TR-FRET data are typically calculated and presented ratiometrically using the following formula:

   [(665 nm/615 nm) x 1,000]

2. Calculate the TR-FRET ratio for each well. Alternatively, the signals at 665 nm can be used directly to analyze the data.
3. Create a standard curve by analyzing the data according to a nonlinear regression using the four-parameter logistic equation (sigmoidal dose-response curve with variable slope) and a $1/Y^2$ data weighting. For correct analysis, the highest standard point should be removed from the curve if it is present after the hook point (*i.e.*, if it shows lower counts compared to the next standard).

Performance Characteristics

Representative Data

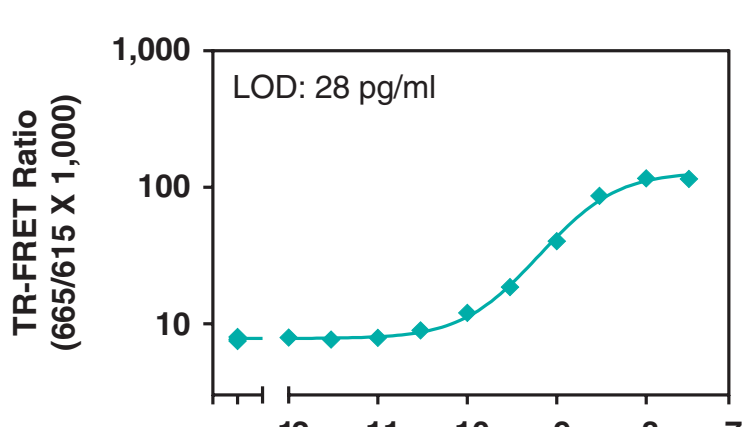

Figure 3. Typical standard curve using lamp excitation Data represent the mean ± standard deviation of triplicate measurements (three wells) for each standard dilution. The data was generated using a low-volume 384-well white plate read with an EnVision® Multilabel Plate Reader equipped with a TR-FRET option.

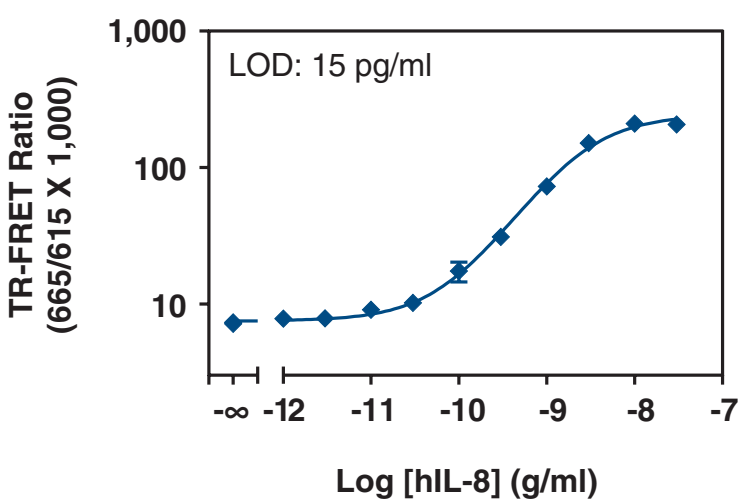

Figure 4. Typical standard curve using laser excitation Data represent the mean ± standard deviation of triplicate measurements (three wells) for each standard dilution. The data was generated using a low-volume 384-well white plate read with an EnVision® Multilabel Plate Reader equipped with a TR-FRET option.

Sensitivity

The limit of detection (LOD) was calculated by adding two standard deviations to the mean background counts of 12 zero-standard replicates and calculating the corresponding concentration on the standard curve.

The lower limit of quantification (LLOQ) was calculated by adding 10 standard deviations to the mean background counts of 12 zero-standard replicates and calculating the corresponding concentration on the standard curve.

	Assay Buffer 1 (1X)		DMEM + 10% FBS		RPMI + 10% FBS	
	Laser	Lamp	Laser	Lamp	Laser	Lamp
# of Standard Curves	13	13	3	3	3	3
Standard Curves LOD (pg/ml)	15	27	27	66	17	37
LLOQ (pg/ml)	74	122	122	268	80	192

Table 3. Sensitivity of the IL-8 (human) TR-FRET Assay Kit

Precision

Intra-assay precision:

Three samples of known concentration (low, medium, high) were tested twenty times each on the same plate to assess intra-assay precision. The assay was run in a 384-well plate with the standards and samples prepared in Assay Buffer 1 (1X). The resulting low %CV (<10%) for each sample indicates good reproducibility within an assay.

	Sample Concentration		
	Low	Medium	High
# of Samples	20	20	20
Mean (pg/ml)	366	1,009	3,655
SD (pg/ml)	18	60	256
CV (%) Intra-Assay	5.0	6.0	7.0

Table 4. Intra-assay precision

Inter-assay precision:

Three samples of known concentration were tested in separate assays to assess inter-assay precision. A total of seven independent experiments were performed by two operators using two different kit lots: The assay was run in 384-well plates with the standards and samples prepared in Assay Buffer 1 (1X) and tested in triplicate. The resulting low %CV (<10%) for each sample indicates good reproducibility between assays.

	Sample Concentration		
	Low	Medium	High
# of Samples	20	20	20
Mean (pg/ml)	321	977	3,794
SD (pg/ml)	27	74	214
CV (%) Intra-Assay	8.3	7.6	5.7

Table 5. Inter-assay precision

Recovery

To assess the accuracy of the assay, spike-to-recovery experiments were conducted using Assay Buffer 1 (1X) and culture media. Each assay consisted of one standard curve and three spike concentrations (low, medium, high) assayed in triplicate in Assay Buffer 1 (1X), DMEM (supplemented with 10% FBS), or RPMI (supplemented with 10% FBS). The percent recovery is reported as the mean concentration of nine wells determined using a standard curve prepared in the corresponding diluent. The data shows recovery values within the range of 80-120% demonstrating assay accuracy.

	Spike Level	Expected (pg/ml)	Observed (pg/ml)	% Recovery
Assay Buffer 1 (1X)	High	3,000	2,919	97
	Medium	1,000	827	83
	Low	300	245	82
DMEM + 10% FBS	High	3,000	2,912	97
	Medium	1,000	1,004	100
	Low	300	276	92
RPMI + 10% FBS	High	3,000	2,741	107
	Medium	1,000	691	81
	Low	300	207	81

Table 6. Recovery in assay buffer or culture media

Linearity

To assess the linearity of the assay, a high concentration of IL-8 was spiked into culture medium supplemented with 10% FBS and diluted with the same culture medium with 10% FBS. The assay was run in a 384-well plate with standards prepared in the same type of supplemented culture medium. Each sample was measured in triplicate. Data shows recovery values within 80-120% confirming assay linearity.

	Expected (pg/ml)	Observed (pg/ml)	% Recovery
DMEM + 10% FBS	3,750	4,341	116
	1,875	1,928	103
	938	957	102
	469	510	109
	234	276	118
RPMI + 10% FBS	1,875	2,108	112
	938	980	104
	469	520	111
	234	253	108
	117	134	114

Table 7. Dilutional linearity in culture medium

Assay Summary		
Assay Type	Homogeneous sandwich immunoassay	
Format	384-well plate	
Hands-on Time	15 minutes	
Incubation Time	1 hour	
Sample Type (Volume)	Cell culture supernatant (15 µl)	
Specificity	Native and recombinant human IL-8	
	Laser excitation	Lamp excitation
Limit of Detection (LOD)	15 pg/ml	28 pg/ml
Lower Limit of Quantification (LLOQ)	74 pg/ml	122 pg/ml
EC_{50} Value	1.9 ng/ml	2.1 ng/ml
Dynamic Range	15-10,000 pg/ml	28-10,000 pg/ml

Table 8. Assay validation summary for the IL-8 (human) TR-FRET Biomarker Assay Kit

RESOURCES

References

1. Gonzalez-Aparicio, M. and Alfaro, C. Influence of interleukin-8 and neutrophil extracellular trap (NET) formation in the tumor microenvironment: Is there a pathogenic role? *J. Immunol. Res.* **6252138** (2019).
2. Sharma, I., Singh, A., Siraj, F., et al. IL-8/CXCR1/2 signalling promotes tumor cell proliferation, invasion and vascular mimicry in glioblastoma. *J. Biomed. Sci.* **25(1)**, 62 (2018).
3. Ha, H., Debnath, B., and Neamati, N. Role of the CXCL8-CXCR1/2 axis in cancer and inflammatory diseases. *Theranostics* **7(6)**, 1543-1588 (2017).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.