FluoroCet

FCET96A-1

User Manual

See Package Contents for Storage Temperatures

Version 3
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Contents

Product Description

The FluoroCet exosome quantitation kit is a fluorescence-based, highly sensitive enzymatic assay that measures esterase activity present inside exosomes. The assay is active with exosomes from all mammalian species tested (Human, Mouse, Rat) and is compatible with exosomes isolated using ExoQuick, ExoQuick-TC, ultracentrifugation, immunoaffinity purification and chromatography methods. A standard curve that has been calibrated to isolated exosomes by NanoSight analysis is included in the kit. The FCET96A-1 kit contains all of the necessary reagents to perform 96 reactions.

List of Components

COMPONENT	AMOUNT	STORAGE
Buffer A	5.5 mL	-20°C
Buffer B	5.5 mL	-20°C
Detection reagent	1 Vial	-20°C
5X Reaction Buffer	20 mL	-20°C
Acetylcholine chloride	1 Vial	-20°C
FLUOROCET Standard	15 µL	-20°C
Exosome Lysis Buffer	5 mL	+4°C
Opaque 96 well microtiter plate	1	RT

Storage

The kits are shipped on blue ice and the individual components must be stored at the indicated temperature upon receipt. Properly stored kits are stable for 6 months from the date received.

Important Information

Since the FluoroCet kit is a fluorescence-based detection kit, you MUST use the included opaque 96 well microtiter plate for both calibration of the instrument and for running the samples. Use of other plates can affect the sensitivity of the detection. **The kit is designed to be used once for calibration, and once for running samples. If you wish to purchase more opaque plates, ordering information for this is listed below.**

Protocol:

Equipment to be supplied by the user

1. Fluorescence plate reader
2. Multichannel pipets (recommended)
3. DI water
4. Opaque 96 well plates, SigmaCat #: CLS3916-100EA (optional, one included in kit)

A. Instrument Calibration

Based on the variability of detection/sensitivity settings for different plate readers, we recommend running an instrument setting calibration prior to the onset of your experiment. The signal from this assay can be robust, and it is best to use a detector setting that is either "automatic" or medium sensitivity. Please note that the RFU (relative fluorescence units) values you observe for your samples will vary depending on the instrument settings and reaction time. We have the following guidelines for optimization. Please refer to the documentation of your instrument for more information on its settings:

1. Prepare standard curve as described in the protocol below (B.6. Standard preparation and 7. Assay Protocol for the Standards)

2. Acquire readings at time points 5, 10 and 20 min using automatic exposure settings (if available on your instrument) or manually set the detector to medium sensitivity.

3. Choose the collection time that provides a good signal over the background and a linear standard curve.

4. If you observe non-linearity at low standard concentrations, choose a longer assay time to collect a stronger signal. If you observe non-linearity at high standard concentrations, choose a shorter assay time to avoid detector saturation. If saturation is observed at a medium setting, a low sensitivity setting may be used.

Note: Sample data that are provided below for guidance (Example Data and Applications). You may observe RFU values that vary from these due to your instrument settings. You data may be valid with RFU values outside what is shown with our sample data. Please examine your data for linearity and signal detection at the high and low ends of your standard curve.

5. Proceed with analysis of the experimental samples.

B. Experimental procedure

1. Exosome sample preparation:

a) Use approximately 250-500ng protein equivalent of input exosomes/well for quantitation via FluoroCet.

b) Assay this protein amount by Nanodrop or BCA assay of the exosomes isolated by ExoQuick (see Step 2 below) or other exosome isolation method.

2. Exosome precipitation with ExoQuick/ExoQuick-TC

a) If frozen, thaw bio-fluids on ice.

b) Centrifuge @ 3000 x g for 15 minutes to remove cells and cell debris.

c) Transfer supernatant to a sterile vessel and add the appropriate volume of ExoQuick or ExoQuick-TC.

d) Mix well by inversion 3x.

e) Place at 4°C for 30 minutes to overnight according to the table below.

f) Centrifuge at 1500 x g for 5 minutes to remove all traces of fluid (don't disturb the pellet).

Incubation time at 4°C	**Bio-fluid**	**Sample volume**	**ExoQuick Volume**	**ExoQuick-TC volume**
30 minutes	Serum	250µL	63µL	none
Overnight	Ascites fluid	250µL	63µL	none
Overnight	culture media	5mL	none	1mL
Overnight	Urine	5mL	none	1mL
Overnight	spinal fluid	5mL	none	1mL

3. Reagent Setup

a) Buffer A: Thaw and aliquot into desirable volume, single-use vials. 1mL Buffer A is sufficient for 20 wells. Store aliquots at -80°C.

b) Buffer B: Thaw and aliquot into desirable volume, single-use vials. 1mL Buffer B is sufficient for 20 wells. Store aliquots at -80°C.

c) Detection reagent: Reconstitute by adding 110 µL DMSO. Aliquot into single-use vials. Freeze at -80°C.

d) 5X Reaction Buffer: Dilute 1:5 in DI water to make a 1X solution. Store the 1X solution at 4°C.

e) Acetylcholine Chloride: Make a stock 0.5M solution by weighing out 9.1 mg Acetylcholine Chloride and adding it to 100 µL DI water. A stock vial of Acetylcholine Chloride powder is provided and this should be use to weigh out the 9.1 mg. This solution should be prepared ***fresh*** just prior to performing the assay.

f) Standard: Thaw and aliquot into single-use vials (2 µL required for a single column of standards). Store aliquots at -80°C.

g) Lysis Buffer: Store at 4°C.

4. Buffer preparation

a) Thaw Buffers A and B in the dark and let them equilibrate to room temperature before starting the assay.

b) Working Stock of Buffer A: For every 1 mL of Buffer A, add 10 µL of Detection Reagent. Mix well and keep protected from light. This solution can aliquoted into single use vials and stored for several weeks at -80°C.

c) Working Stock of Buffer B: For every 1 mL of Buffer B, add 4 µL of 0.5M Acetylcholine Chloride solution.

5. Sample preparation

a) Resuspend exosome pellet in PBS.

b) Quantify the total exosomal protein content by Bradford assay or estimate by Nanodrop. Aliquot 600ng- 1µg protein equivalent of exosomes in a maximum volume of 60 µL (sufficient for duplicate samples) in a clean 1.5mL Eppendorf tube. 600ng exosome input for duplicate samples would result in ~250ng exosomes per well.

c) Add 60 µL Lysis Buffer to 60 µL of the exosome suspension from Step 2 to bring to a total of 120 µL.

d) Incubate on ice for 30 mins to liberate exosome proteins.

6. Standard preparation

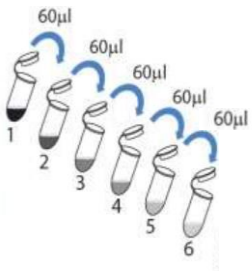
A standard curve should be prepared for each assay. Buffers should be at room temperature before beginning.

a) Dilute FluoroCet standard 1:64 in 1X Reaction buffer in a microcentrifuge tube first (for example, add 2µL Standard to 126µL 1X Reaction Buffer). Vortex to mix well. Use this dilution as the First Standard for the standard curve.

b) Perform serial dilutions of the First Standard in 1X Reaction buffer in microcentrifuge tubes. Vortex to mix well after each dilution.

c) Suggested dilutions for making the FluoroCet standard curve are shown below. To run the standards in duplicate, double the recipes listed and split into two separate wells.

Tube	Exosome Abundance	Dilution Factor	Standard	1X Reaction Buffer
1	2.00E+08	1	128µL	0
2	1.00E+08	1:2	60µL (from tube 1)	60µL
3	5.00E+07	1:4	60µL (from tube 2)	60µL
4	2.50E+07	1:8	60µL (from tube 3)	60µL
5	1.25E+07	1:16	60µL (from tube 4)	60µL
6	6.25E+06	1:32	60µL (from tube 5)	60µL
Blank	0	Blank	0	60µL



7. Assay Protocol

a) For each well of the opaque 96 well plate, add the following:

50µL Standard or lysed exosome sample

+ 50µL Working stock of Buffer A

+ 50µL Working stock of Buffer B

Total: 150µL reaction volume

b) Mix the reagents by gently tapping the sides of the plate.

c) Incubate the plate, protected from light for 20 minutes at room temperature.

d) Read the plate using a fluorescence plate reader immediately at Excitation: 530-570nm and Emission: 590- 600nm. **Premix for 30 sec before reading**. Quantitate the results according to the settings calculated in A. Instrument Calibration.

e) Analyze the data using standard data analysis software, such as Excel that can provide a linear regression for the standard curve. Subtract the blank from your standards and experimental values. The standard curve determines the number of exosomes (not the concentration) of exosomes in the sample. To find the concentration of exosomes in your experimental samples, divide the exosome number in that well by the volume of exosomes that was analyzed.

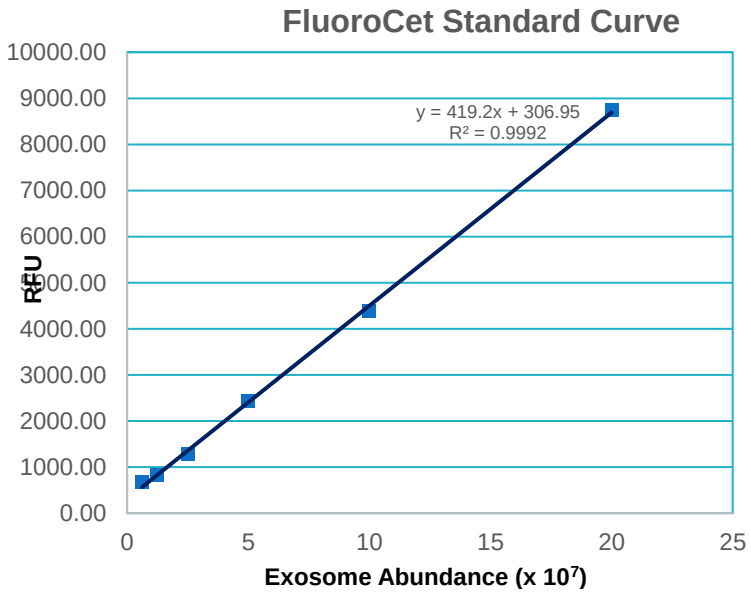
Next Steps and Related Products

Application	Related Products	Website links
Precipitation of Exosomes from other biological fluids		
Exosome Isolation from Tissue Culture Media	ExoQuickTC	https://www.systembio.com/microrna-research/exoquick-exosomes/ordering
Exosome Isolation from Plasma	ExoQuick Plasma prep and Exosome precipitation kit	https://www.systembio.com/microrna-research/exoquick-exosomes/ordering
Protein Characterization of Exosomes		
Western blotting	Exosome antibodies	https://www.systembio.com/microrna-research/exosome-antibody/exoab
Antibody Arrays	ExoCheck Assays	https://www.systembio.com/microrna-research/exosome-antibody-arrays
ELISA	ExoELISA Kits	https://www.systembio.com/microrna-research/exosome-antibody/elisas
Quantification of Exosomes		
Quantification of exosomes	EXOCET Assays	https://www.systembio.com/microrna-research/exosome-antibody/exocet-assay
RNA extraction from Exosomes		
RNA extraction and profiling	SeraMir kits	https://www.systembio.com/microrna-research/seramir-exosome-rna-profiling/overview

Example Data and Applications

These data were taken using a SpectraMAX Gemini XS from Molecular Devices using Automatic PMT settings. Your RFU values will vary depending on the instrument settings and your reaction time. The sample data below have the background subtracted.

Exosome Abundance	x10^7	Average RFU
2.00E+08	20	8750.63
1.00E+08	10	4379.08
5.00E+07	5	2427.27
2.50E+07	2.5	1280.56
1.25E+07	1.25	828.59
6.25E+06	0.625	681.42



Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site: **http://www.systembio.com**

For additional information or technical assistance, please call or email us at:

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- This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

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