EXOCET Exosome Quantitation Assay

EXOCET96A-1

User Manual

Check Package Contents for Storage Temperatures

Version 4
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Contents

Product Description

The EXOCET exosome quantitation kit is designed as a direct measurement of esterase activity known to be within exosomes. The EXOCET 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 chromatographic methods. The EXOCET assay is an enzymatic, colormetric assay read at OD405. The assay is rapid, only 20 minutes from start to finish and quantitative. A standard curve that has been calibrated to isolated exosomes by NanoSight analysis is included in the kit. The EXOCET96A-1 kit contains all of the necessary reagents to perform 96 reactions.

List of Components

COMPONENT	AMOUNT	STORAGE
Exosome Lysis Buffer	**2 mL**	**+4°C**
EXOCET Buffer A	**5 mL**	**+4°C**
EXOCET Buffer B	**50 µL**	**-20°C**
PBS-B Buffer (sterile)	**5 mL**	**+4°C**
EXOCET Standard	**400 µL**	**-20°C**
96 well assay plate (12x8 strips)	**1**	**+4°C**

Storage

The kits are shipped on blue ice and the individual components should be stored according to the recommendations in the List of Components upon receipt. Properly stored kits are stable for 1 year from the date received.

General Information

The materials provided in the EXOCET kit are designed such that the user can use the kit once for calibration under their laboratory conditions and equipment and a second time for the running of samples. Further usage may result in error as freeze-thawing of the components may result in reduced performance.

The standard curve in the EXOCET assay is calibrated to the signal observed from a certain number of exosomes as measured by NanoSight (NTA) analysis. The standards do not contain exosomes as these are not reproducibly stable for shipping and storage for the 1 year shelf life of the assay. The standard curve measures the number of exosomes in a sample, this can easily be used to find the concentration of exosomes in your sample. This kit has been validated against Nanosight (NTA) quantitated exosomes.

Protocol

Equipment to be supplied by the user

1. Microtiter plate sealing film/cover
2. Microtiter plate spectrophotometer with 405nm absorbance capability
3. Multichannel pipets (recommended)
4. Standard PBS buffer (1x)

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 absorbance 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 (EXOCET Standard Curve and EXOCET Assay Sections)

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 (Sample Data). You may observe absorbance values that vary from these due to your instrument settings. You data may be valid with absorbance 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

Exosome sample preparation

Precipitate exosomes according to your usual protocol.

This protocol works best from a frozen or fresh exosome pellet or in a highly concentrated solution (about 10^7 exosomes/µL, corresponds to a protein concentration of 2µg/µL). Add approximately 20-100 µg exosomal protein per reaction. You can do a protein assay with a small aliquot of the precipitated exosomes to determine how much to use, a BCA assay or a NanoDrop measurement at A280 will estimate the protein in your sample.

After the protein assay, calculate the volume of exosomes needed for 20-100 µg protein. **Add Lysis Buffer to a total volume of 100 µl.** (We usually use 20 µl of exosomes and 80 µl of Lysis Buffer.)

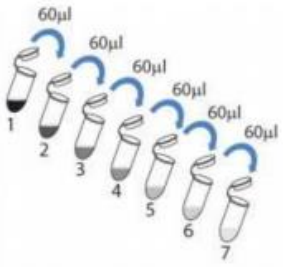
1. Incubate at 37°C for 5 minutes to liberate exosome proteins
2. Vortex for 15 seconds
3. Centrifuge at 1500 x g for 5 minutes to remove debris
4. Transfer supernatant to new centrifuge tube on ice
5. Exosome protein samples are now ready to be assayed on the microtiter plate

EXOCET Standard Curve

A standard curve should be prepared each time the assay is performed. Bring all buffers to room temperature before beginning.

1. Dilute EXOCET standard by performing serial dilutions with PBS-B buffer in microcentrifuge tubes first.
2. Suggested dilutions for making the EXOCET standard curve are shown below. If you want to run the standards in duplicate, double the amounts listed and split into two separate wells.

Tube	Exosome Abundance (# of exosomes)	Dilution Factor	Standard	PBS-B Buffer
1	1.28E+10	1	128µL	0
2	6.40E+09	1:2	60µL (from tube 1)	60µL
3	3.20E+09	1:4	60µL (from tube 2)	60µL
4	1.60E+09	1:8	60µL (from tube 3)	60µL
5	8.00E+08	1:16	60µL (from tube 4)	60µL
6	4.00E+08	1:32	60µL (from tube 5)	60µL
7	2.00E+08	1:64	60µL (from tube 6)	60µL
Blank	0	Blank	0	60µL



EXOCET Assay

1. Prepare the EXOCET Reaction Buffer **fresh** just before using by combining **Buffer A** with **Buffer B** depending upon the number of reactions you are preparing. You will need to use 50 µL of Buffer A plus 0.5 µL Buffer B per reaction. Create an amount of EXOCET Reaction Buffer that is enough for your assay mix thoroughly and use within 1 hour.

2. In each clear well in the 96 well plate:

First add, 50 µL of Reaction buffer (A+B made fresh)

Then add, 50 µL of Standard or Exosome sample

Total: 100 µL reaction volume

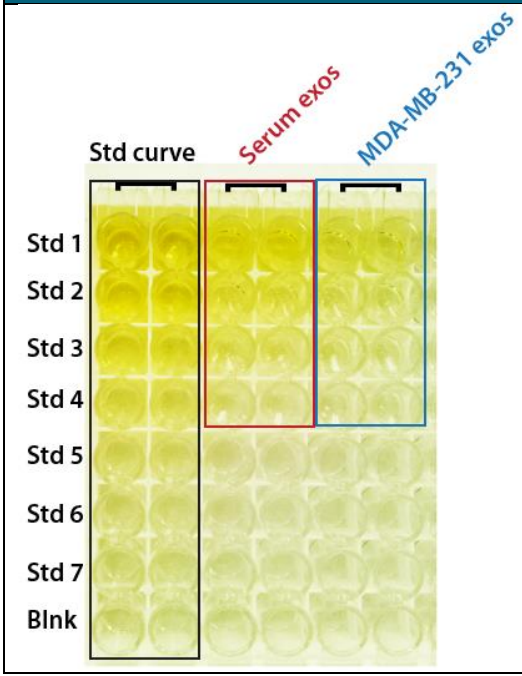
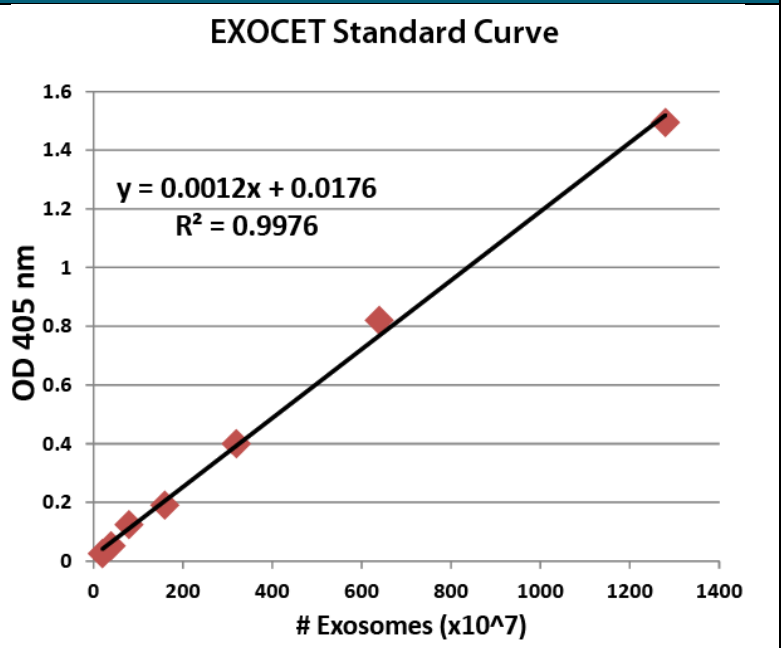
3. Let the plate incubate for 10-20 minutes at room temperature. (Use the time you determined to be optimal during the calibration step in this protocol).

4. Read plate using a spectrophotometric plate reader immediately at 405 nm (no reaction stop buffer required).

5. Quantitate results by calculating the standard curve and plotting the sample readings on the standard curve. The Sample Data shows an example data set. The absorbances measured for each standard may vary based on incubation time. The standard curve gives the number (not concentration) of exosomes in your sample. You should calculate the concentration in your sample by dividing the number of exosomes in the sample by the volume of the exosome sample used in the assay.

Sample Data

# Exos	x10^7	Avg of OD405
1.28E+10	1280	1.494
6.40E+09	640	0.820
3.20E+09	320	0.3995
1.60E+09	160	0.1905
8.00E+08	80	0.1235
4.00E+08	40	0.0515
2.00E+08	20	0.0245

Photo of Plate with Standards and Exos	Standard Curve from data
	

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