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PRODUCT	**EXOCET Exosome Quantitation Assay Kit**
CATALOG #	**EXOCET96A-1**
REACTIVITY	**All species exosomes**
VERSION	**09-18-2015**
STORAGE	**See Package contents section below**
SHELF LIFE	**12 months from date of receipt with proper storage**
SHIPPING	**Blue Ice**

PACKAGE CONTENTS

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

DESCRIPTION

The EXOCET exosome quantitation kit is designed as a direct measurement of esterase activity known to be within exosomes (1, 2). 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 chromatography methods. The EXOCET assay is an enzymatic, colormetric assay read at OD405. The assay is rapid, only 20 minutes from start to finish and very 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.

1. Savina A, Vidal M, Colombo MI. *The exosome pathway in K562 cells is regulated by Rab11*. J Cell Sci. 2002 Jun 15;115(Pt 12):2505-15.

2. Gupta S, Knowlton AA. *HSP60 trafficking in adult cardiac myocytes: role of the exosomal pathway*. Am J Physiol Heart Circ Physiol. 2007 Jun;292(6):H3052-6. Epub 2007 Feb 16.

EXOCET PROTOCOL

I. Equipment to be supplied by user

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

II. PROCEDURE

a. Protocol works best from a frozen exosome pellet or in a highly concentrated solution (about 10^7 exosomes/ul, 2ug/ul). Add approximately 20-100 ug exosomes per reaction.

b. Exosome precipitation with ExoQuick/ExoQuick-TC

1. If frozen, thaw sample on ice.
2. Centrifuge @ 3000x g for 15 minutes to remove cells and cell debris
3. Transfer supernatant to a sterile vessel and add the appropriate volume of ExoQuick or ExoQuick-TC

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

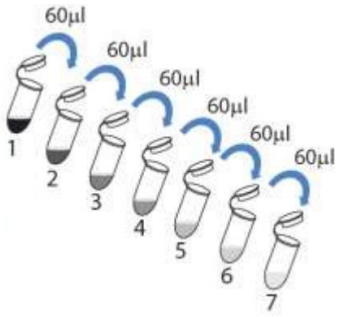
4. Mix well by inversion 3x
5. Place at 5°C from 30 minutes to overnight according to the table
6. Centrifuge at 1500 x g for 5 minutes to remove all traces of fluid (don't disturb the pellet)
7. For exosome pellets: Add 1 mL of standard PBS buffer (not PBS-B in kit) to exosome pellet and vortex 15 seconds to resuspend the exosomes. For exosomes in suspension, use 20 ul (need at least 50 ug exosome proteins) and to this add 80 ul Exosome Lysis Buffer. This makes two 50 ul duplicates
8. Incubate at 37°C for 5 minutes to liberate exosome proteins
9. Vortex for 15 seconds
10. Centrifuge at 1500 x g for 5 minutes to remove debris
11. Transfer supernatant to new centrifuge tube on ice
12. Exosome protein samples are now ready to be assayed on the microtiter plate

III. 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, then simply double the recipes listed and split into two separate wells.

Tube	# Exosomes	Dilution Factor	EXOCET standard	PBS-B buffer
1	1.28E+10	1	120 ul	0
2	6.40E+09	1:2	60 ul (from tube 1)	60 ul
3	3.20E+09	1:4	60 ul (from tube 2)	60 ul
4	1.60E+09	1:8	60 ul (from tube 3)	60 ul
5	8.00E+08	1:16	60 ul (from tube 4)	60 ul
6	4.00E+08	1:32	60 ul (from tube 5)	60 ul
7	2.00E+08	1:64	60 ul (from tube 6)	60 ul
blank	0.00E+00	blank	0	60 ul



V. EXOCET Assay

1. Prepare the EXOCET Reaction buffer **fresh** just before using by combining Buffer A with Buffer B depending upon the number of reaction you are preparing. ***Example:*** If you are performing 20 EXOCET reactions, pipet 1 mL of Buffer A into a fresh tube and add 10 ul Buffer B. Mix thoroughly and use within 1-2 hours.

2. In each clear well in the 96 well plate,
   + First add, 50 uL of Reaction buffer ***(A+B made fresh)***
   Then add, 50 uL of Standard or Exosome sample
   Total: 100 uL reaction volume

3. Let the plate incubate for 10-20 minutes at RT (10-20 minutes is optimized and recommended for the standard curve and quantitation of sample exosomes)

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

5. Quantitate results using the sample table of data provided below

VI . Sample Data

Standard curve 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