EV Isolation & Analysis Service

Customer Name: -
Customer Address: -

Customer email:
Customer telephone:

Report No: EVS001

Date Of Sample Receipt: 01/01/2025
Date Of Report: 05/01/2025
Sample Details: HEK293 Conditioned Cell Culture Media
Package Details: Basic Package

Analysed By: SK
Checked By: HN

Service Description

Package Details: basic package

1. Isolation of EV/exosome content using Exo-Spin
2. Measurement of total protein content using a BCA assay
3. Measurement of EV/exosome numbers and size distribution using nano tracking analysis (ZetaView)

Report contents

This report covers the isolation and analysis of extracellular vesicles derived from your provided samples.

Page	Contents
2	Isolation
2	Assay 1: Quantification of Exosomes & Size Distribution
3	Assay 2: Quantification of Protein in Exosome Preparations
4	Assay 3: Isolation & Assay Protocols
5, 6	NTA Report Raw Data
7	Report approval

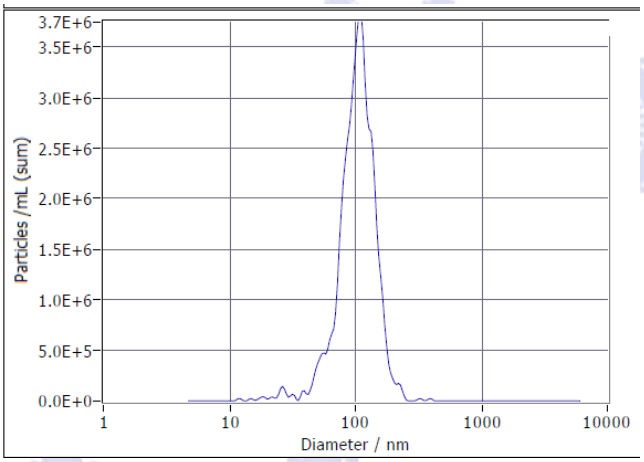
Isolation

50 ml of cell culture media was received and was purified according to the standard Exo-spin protocol using Exo-spin buffer for sample concentration followed by purification on an EX01-mini column. The sample was eluted in 180 µl.

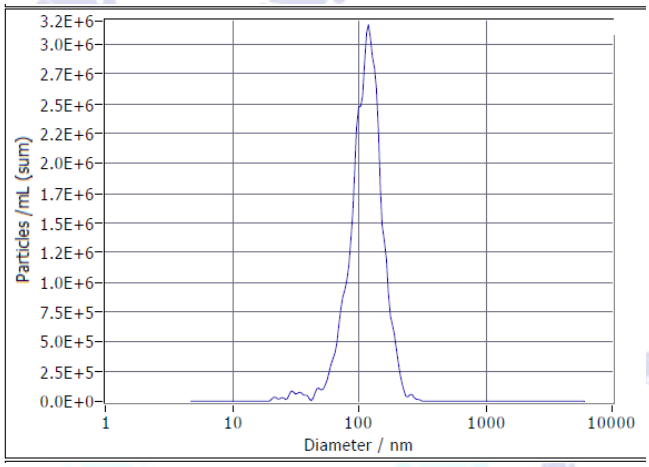
Assay 1: Quantification Of Exosomes & Size Distribution

Method Of Particle Quantification: **ZetaView® NTA Analysis**

Number Of Positions Measured Per Sample: **11 Positions**



Sample Name: X-XXX-1
Total Concentration Of Particles: 3.9E+6/mL
Mean Diameter Size Distribution (nm): 108.2
Observation: Size range consistent with exosome particles



Sample Name: X-XXX-2
Total Concentration Of Particles: 3.2E+6/mL
Mean Diameter Size Distribution (nm): 119.6
Observation: Size range consistent with exosome particles

Note: Raw data and reports generated from the NTA analysis can be found in your report package folder under the name 'NTA Generated Reports'. There you can find each sample's NTA report and more detailed information.

The NTA instrument is calibrated prior to exosome preparation analysis, using a nano-bead standard with a diameter of 100nm. 0.22 µm reagent grade water is used to clean the instrument between each step of analysis.

A detailed explanation of each dataset and an annotated example report are included in the report upon receipt.

Assay 2: Quantification Of Protein in Exosome Preparations

Method Of Protein Quantification: **Bicinchoninic Acid Assay** (**BCA assay**)

Standard curve generation

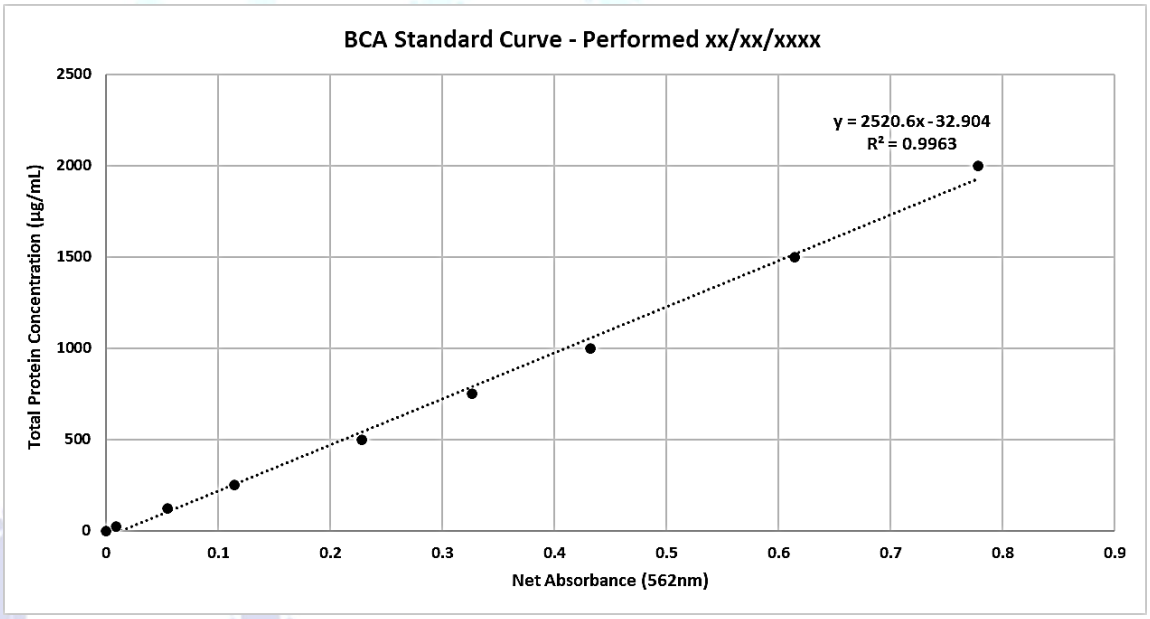


Figure 1. Standard curve produced from BSA protein standard (2 mg/mL). Exosome protein quantification is then derived from this graph.

Note: The standard curve (fig 1.) measured from triplicates of each exosome sample preparation. Blank samples are derived from using 0.22 µm filtered reagent grade water.

Assay sample results:

Sample Name: X-XXX-1
Total Protein Concentration: 125.43 µg/mL

Sample Name: X-XXX-2
Total Protein Concentration: 119.67 µg/mL

Exosome Isolation

The isolation of exosomes is carried out using the Exo-spin™ EX01 protocol. The full protocol can be found on the Exosome product page at www.cellgs.com or by navigating the following page: https://www.cellgs.com/products/exo-spinand8482-mini.html

Quantification Of Exosomes & Size Distribution

Nanoparticle tracking analysis (NTA) is utilised to quantify the mean size and particle number of exosome preparations. The instrument used by the trained technician is the Particle Metrix GmbH ZetaView®. The instrument is calibrated using a 100 nm latex bead standard, readying the instrument to perform measurements.

Isolated exosome preparations are diluted to a compatible range for the instrument, using 0.22 µm filtered, sterile phosphate buffered saline (PBS), before being applied to the instrument for analysis. Automated measurements are taken at 11 positions, providing a broad analysis for each sample, and automatically providing feedback on any errors between each position. Thorough cleaning using 0.22 µm filtered, sterile PBS is carried out between each sample that is processed.

A report is generated for each sample, providing information on the mean size distribution (nm) of particle populations, a total concentration (number of particles per mL of solution) of the particle population, as well as the zeta potential.

Quantification Of Protein in Exosome Preparations

The protein quantification of exosome preparations is carried out utilising a BCA protein assay (ThermoFisher Scientific) and the subsequent absorbance reading using a plate reader (Synergy HT, Biotek, USA).

Exosome preparations are resuspended in 0.22 µm filtered PBS following isolation. From here, 10 µL of each sample is applied in triplicate to a 96-well plate, whilst a BSA standard comprising a dilution series from 2,000 – 20 µg/mL is also applied in to the plate in triplicate, to generate a standard curve. 0.22 µm filtered PBS is applied in triplicate as a negative control.

200 µL of the carbonate and cupric sulphate solution is applied to each well before the plate is incubated at 37˚C for 30 minutes.

The plate is then read using a protocol for absorbance at a wavelength of 562 nm. The data obtained from this read out is then analysed using the standard curve, in order to determine the protein content for each sample.

Sample Name: X-XXX-2

PARTICLEMETRIX
www.particle-metrix.de

Electrophoresis & Brownian Motion
Video Analysis
Laser Scattering Microscopy

Operator (Report): ZetaView
Video Operator: ZetaView

Sample Parameters
Sample Name: M1-1in800
Comment: Sample Remarks0:
Sample Remarks1:
Sample Remarks2:
Electrolyte: PBS
Temperature: 21.16 °C sensed
pH 7.0 entered
Conductivity: 37.54 µS/cm sensed

Instrument Parameters
Laser Wavelength: 488 nm
Filter Wavelength: Scatter

Measurement Parameters
Cell S/N: CA0032-0127b

Measurement Mode: Size Distribution 2 Cycles
11 Positions, 1 Removed for Analysis

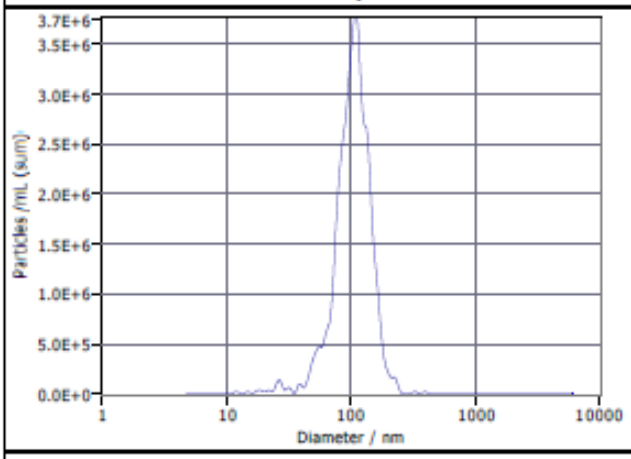
Result (sizes in nm)

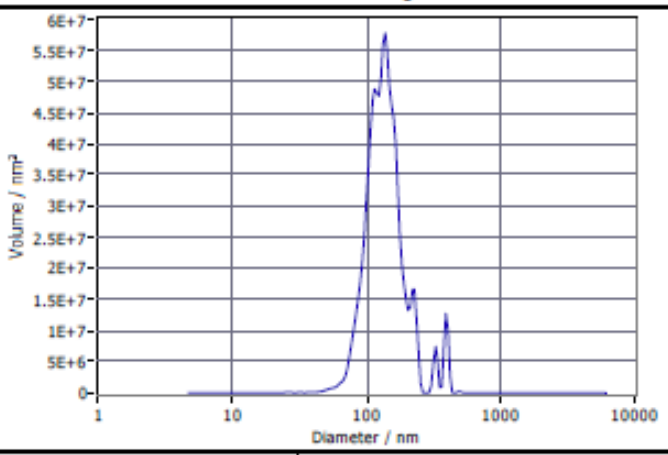
	Number	Concentration	Volume
Median (X50)	103.5	103.5	132.9
Span	35.0	35.0	61.2

Concentration: 7.1E+7 Particles / mL
Dilution Factor: 800
Original Concentration: 5.7E+10 Particles / mL

Quality
Average Counted Particles per Frame: 240
Number of Traced Particles: 1215

Analysis Parameters
Max Area: 1000, Min Area: 10, Min Brightness: 20





Peak Analysis (Concentration)

Diameter / nm	Particles/mL	FWHM / nm	Percentage
108.2	3.9E+6	67.6	100.0

X Values (all sizes are given in nm)

	Number	Concentration	Volume
X10	67.6	67.6	93.0
X50	103.5	103.5	132.9
X90	147.0	147.0	210.9
Span	0.8	0.8	0.9
Mean	107.6	107.6	148.2
StdDev	35.0	35.0	61.2

Comment

(Signature)

Sample Name: X-XXX-1

PARTICLEMETRIX
www.particle-metrix.de

Electrophoresis & Brownian Motion
Video Analysis
Laser Scattering Microscopy

Operator (Report): ZetaView
Video Operator: ZetaView

Sample Parameters
Sample Name: M1-1in800
Comment: Sample Remarks0:
Sample Remarks1:
Sample Remarks2:
Electrolyte: PBS
Temperature: 21.69 °C sensed
pH 7.0 entered
Conductivity: 39.70 µS/cm sensed

Instrument Parameters
Laser Wavelength: 488 nm
Filter Wavelength: Scatter

Measurement Parameters
Cell S/N: CA0032-0127b

Measurement Mode: Size Distribution 2 Cycles
11 Positions

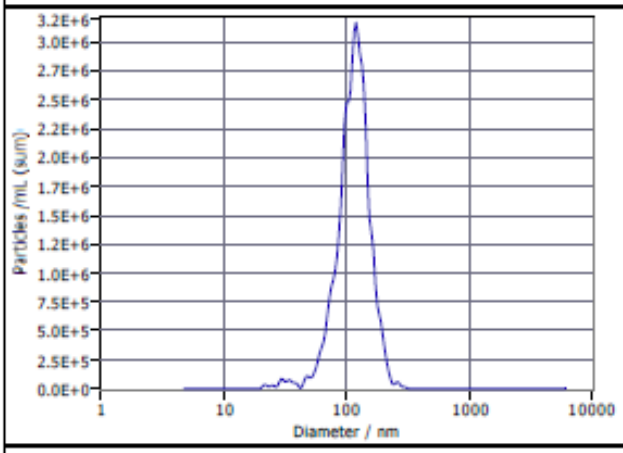
Result (sizes in nm)

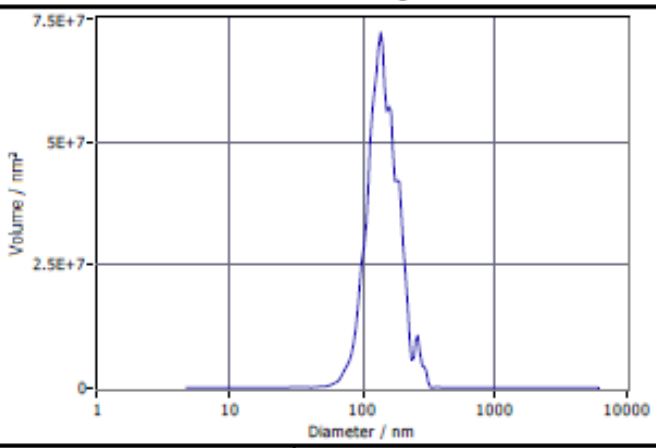
	Number	Concentration	Volume
Median (X50)	113.5	113.5	139.5
Span	35.2	35.2	41.7

Concentration: 5.7E+7 Particles / mL
Dilution Factor: 800
Original Concentration: 4.5E+10 Particles / mL

Quality
Average Counted Particles per Frame: 190
Number of Traced Particles: 1131

Analysis Parameters
Max Area: 1000, Min Area: 10, Min Brightness: 20



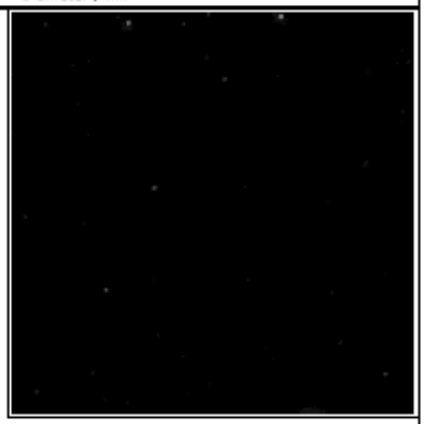


Peak Analysis (Concentration)

Diameter / nm	Particles/mL	FWHM / nm	Percentage
119.6	3.2E+6	62.4	100.0

X Values (all sizes are given in nm)

	Number	Concentration	Volume
X10	74.3	74.3	100.3
X50	113.5	113.5	139.5
X90	159.0	159.0	199.3
Span	0.7	0.7	0.7
Mean	117.4	117.4	149.1
StdDev	35.2	35.2	41.7



Comment

(Signature)

Sample Name: X-XXX-2

Report Approval

Isolation and analysis successfully completed for 2 of 2 samples.

This report has been checked and authorized by

Michael Jones PhD
CEO

Cell Guidance Systems
Maia Building, Babraham Research Campus
Cambridge, CB22 3AT, UK
Tel: +44 (0) 1223 967316
www.cellgs.com