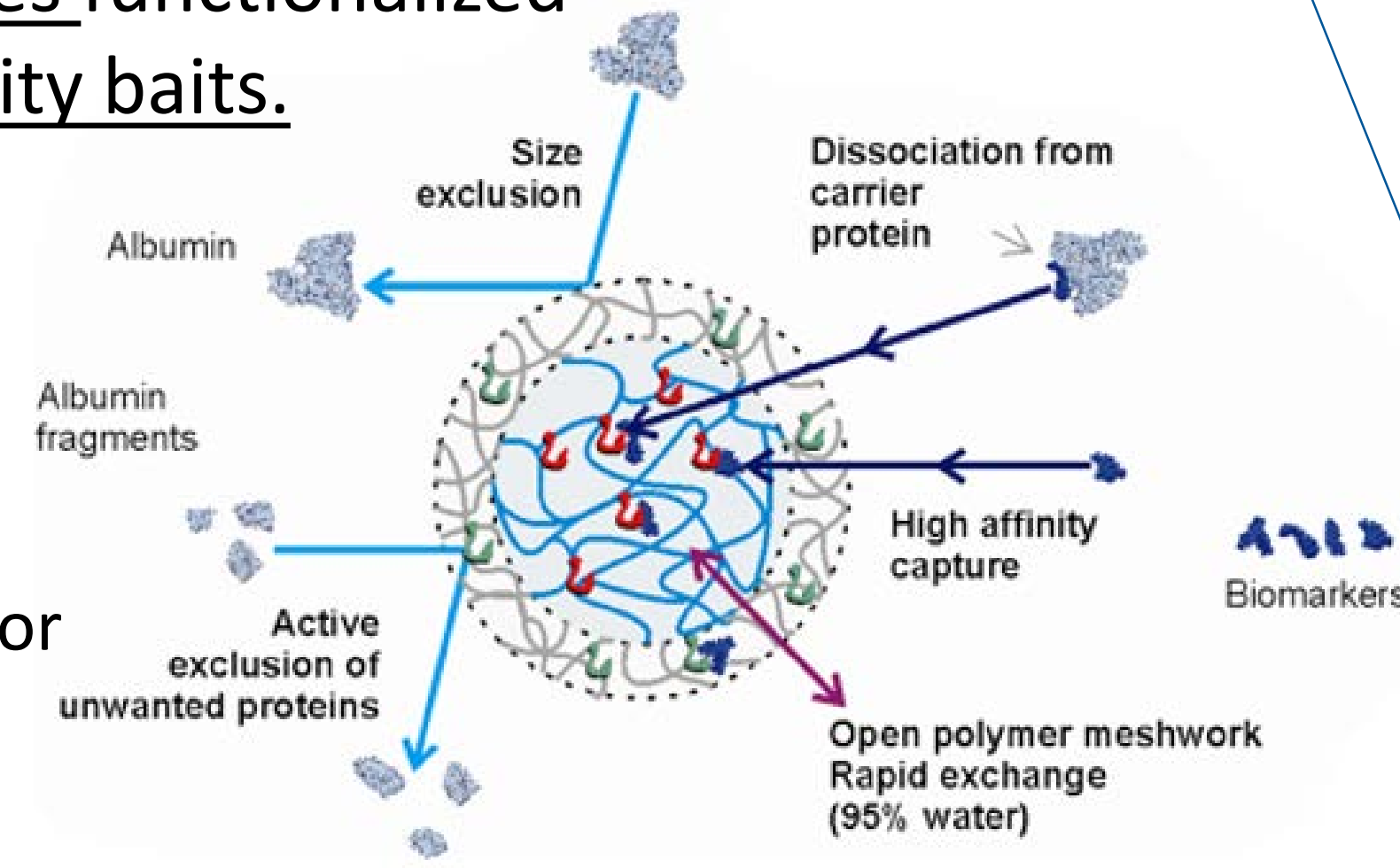


Nanotrap® Particle Technology

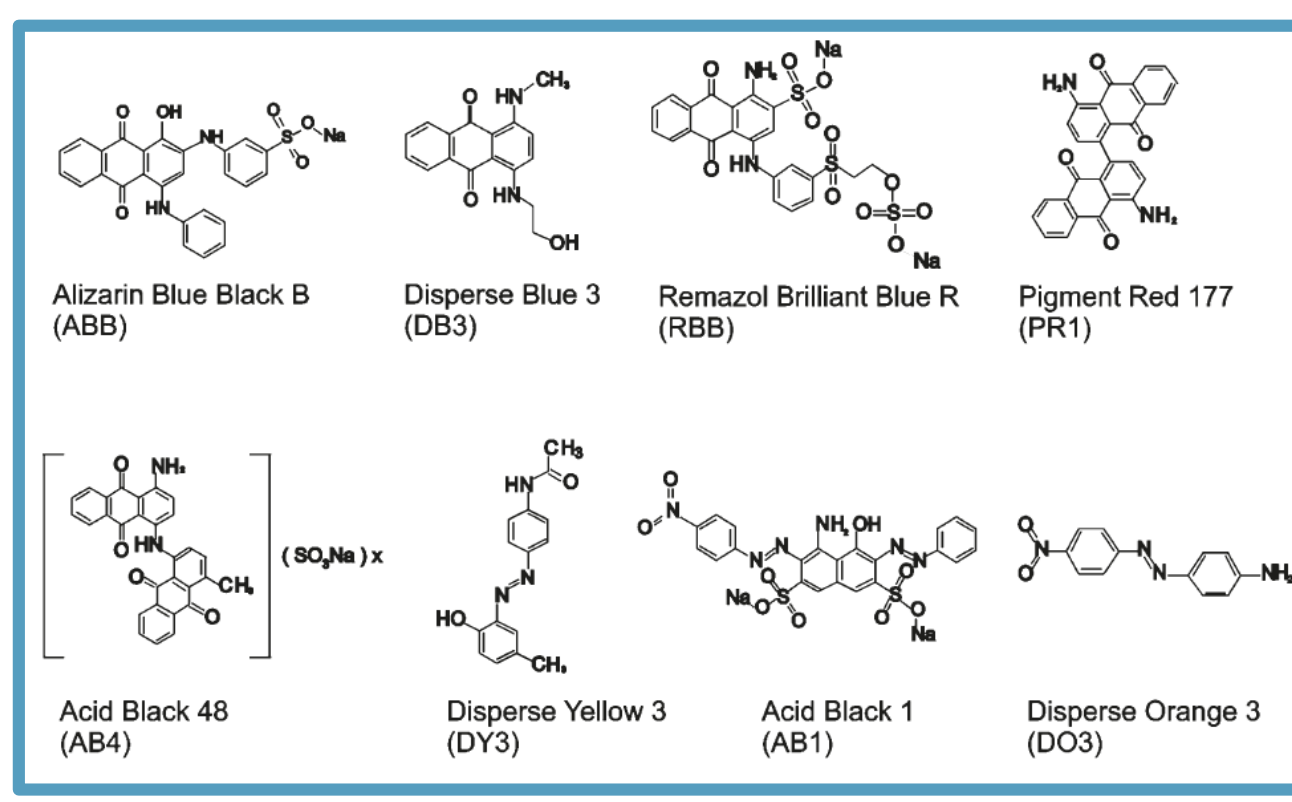
NANOTRAP® PARTICLES

are hydrogel spheres functionalized with chemical affinity baits.

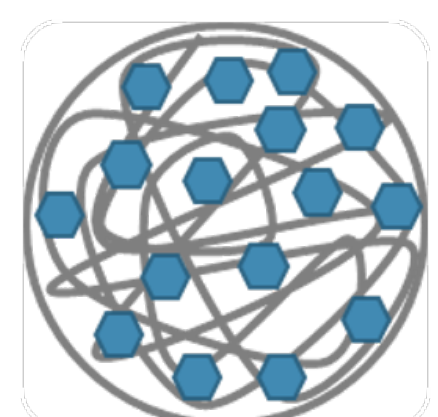
They can **purify, concentrate,** and **preserve** low abundance analytes from any body fluid for a wide array of applications.



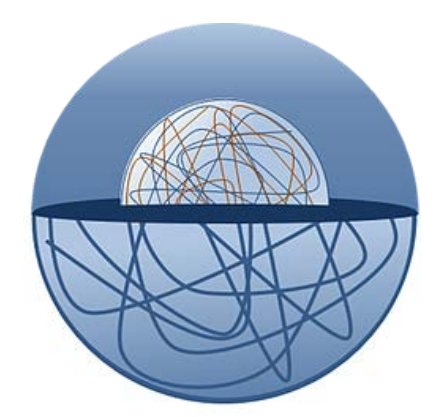
Nanotrap® particle architectures and chemical affinity baits can be altered for different analytes, sample types, and downstream applications



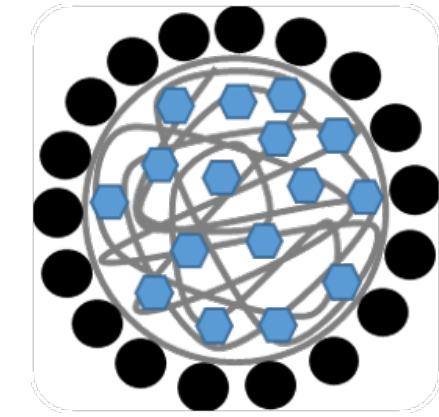
Chemical Affinity Baits



Nanotrap® Core Particles



Nanotrap® Core Shell Particles



Nanotrap® Magnetic Particles

ANALYTES

- Proteins/Peptides
- Metabolites
- Cytokines
- Small Molecules
- Exosomes
- Whole Virus
- Nucleic Acids

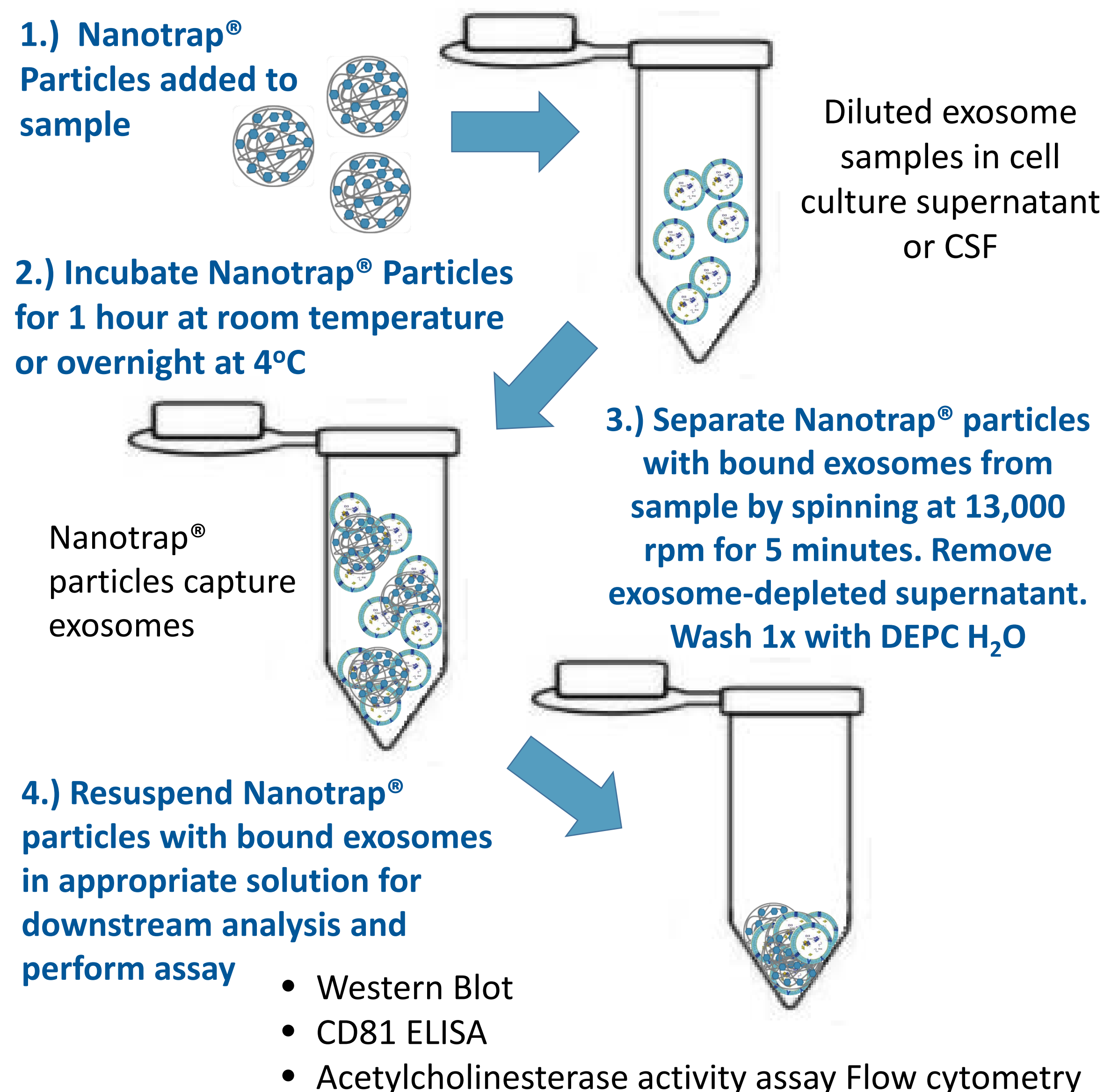
SAMPLE TYPES

- Blood (Whole, Serum, Plasma)
- Urine
- Saliva
- Nasal Fluid
- Sweat
- Cerebrospinal Fluid

APPLICATIONS

- Mass spectrometry
- Immunoassays
- PCR
- Next Gen Sequencing
- Enzymatic assays

Exosome Enrichment with Nanotrap® Particles



- Western Blot
- CD81 ELISA
- Acetylcholinesterase activity assay
- Flow cytometry

Also possible to perform, but not shown in this poster:

- Nucleic acid analysis
- Other enzymatic assays
- Other protein analysis
- Metabolite and cytokine assays

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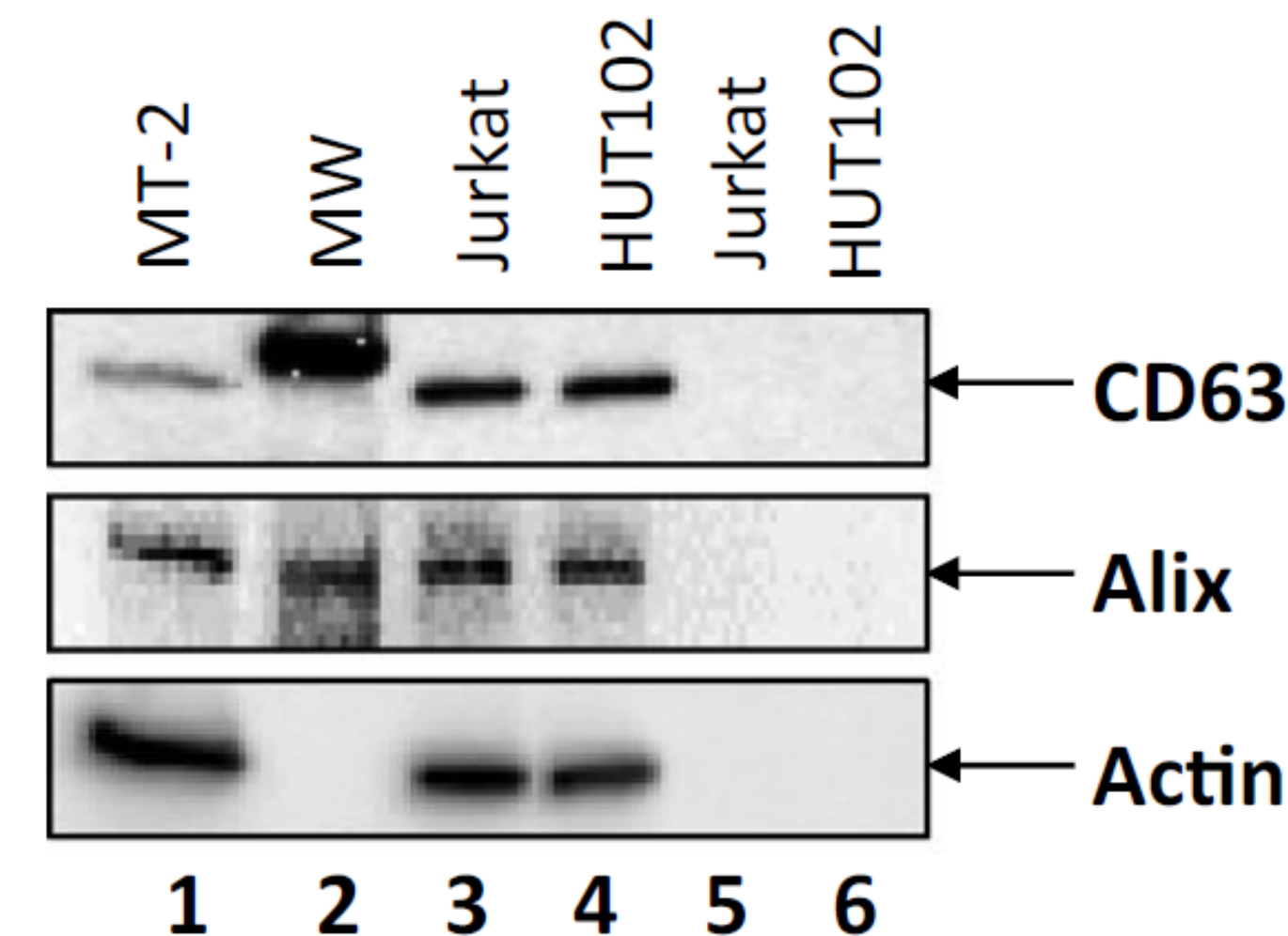
³ George Mason University, Manassas, VA, USA

Overview

This work demonstrates that Nanotrap® particles can be used to enrich exosomes from tissue culture supernatant and cerebrospinal fluid (CSF). Using tissue culture supernatants, Nanotrap® particle enrichment of exosomes was confirmed by Western Blot, CD81 ELISA, acetylcholinesterase (AChE) activity, and flow cytometry. Nanotrap® particle enrichment of exosomes from CSF and PBMCs were confirmed by CD81 ELISA and Western Blot. Finally, using tissue culture supernatants and a CD81 ELISA, it was demonstrated that Nanotrap® particles can be used to enrich exosomes to equal or greater concentrations than exosomes isolated by ultracentrifugation from 10 times the volume of the same starting material.

Nanotrap® Particles Enrich Exosomes from Cell Culture

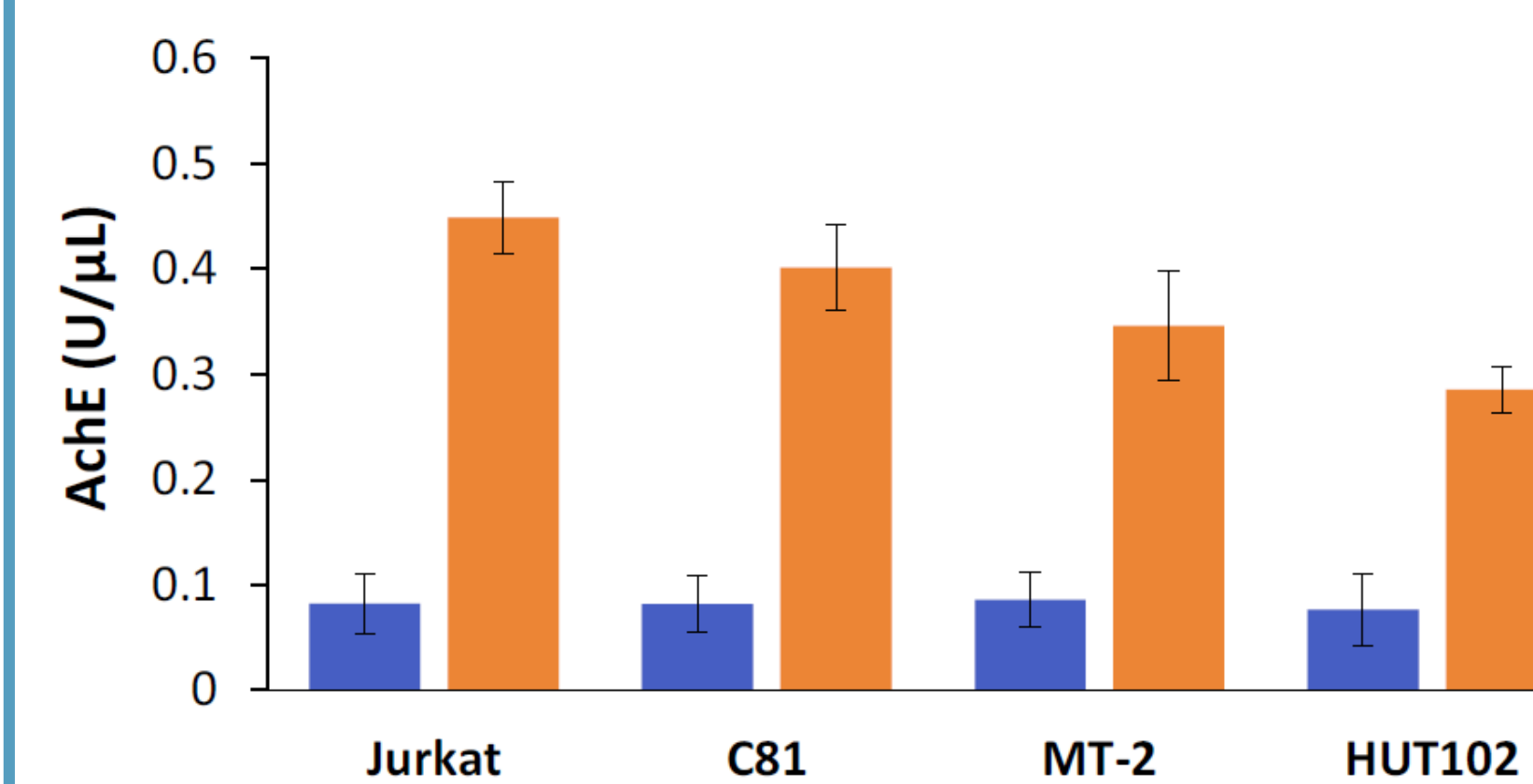
Western blot confirms enrichment of exosomes



Lane 1: MT-2 whole cell extract
Lane 2: Molecular Weight Ladder
Lanes 3 and 4: Tissue culture supernatants processed with exosome capture Nanotrap® particles
Lanes 5 and 6: Tissue culture supernatants processed with control Nanotrap® particles (particles without exosome-binding affinity dye)

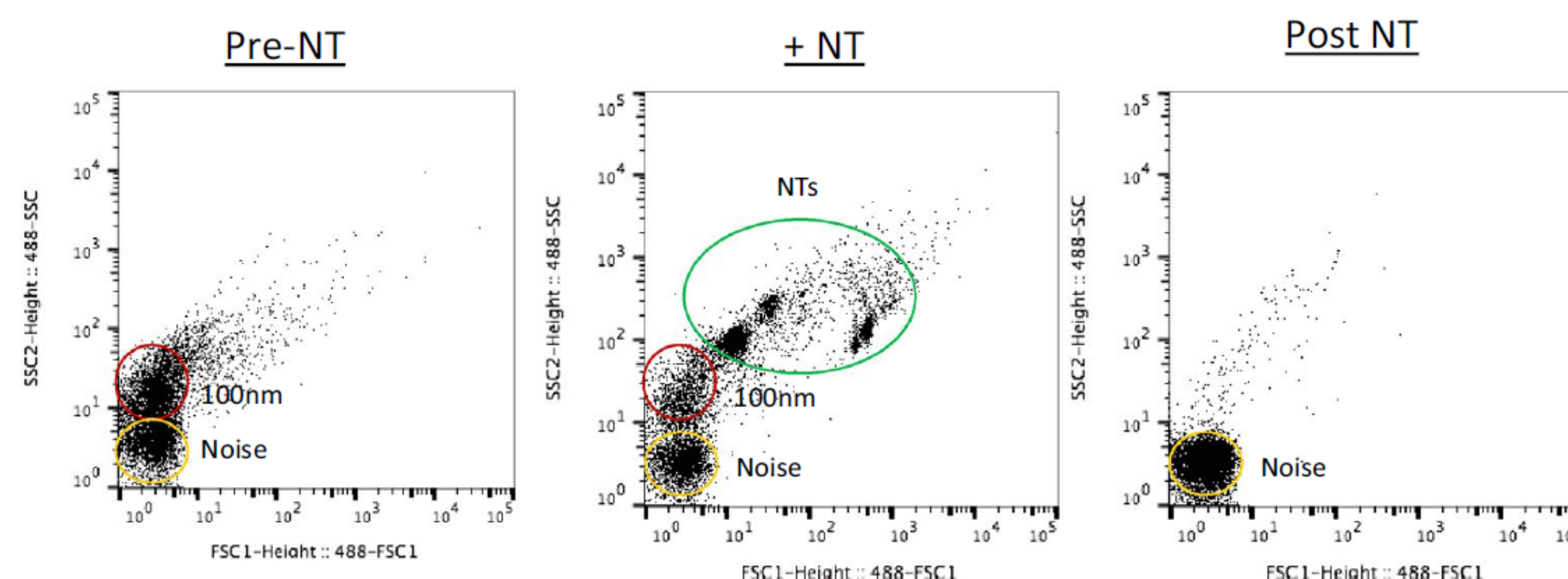
Acetylcholinesterase activity assay confirms enrichment of exosomes

- AChE activity was measured in tissue culture supernatants (blue bars)
- Exosomes were enriched using Nanotrap® particles and AChE activity was measured in the enriched sample (orange bars)



nanoFACS confirms capture of exosomes by Nanotrap® particles

- The population of 100 nm vesicles in the tissue culture supernatant (Pre-NT) shifts on both the forward and side scatter axes when the Nanotrap® particles are added (+ NT), indicating an increase in size.
- After the Nanotrap® particles are removed (Post NT), the dense population of 100 nm vesicles visible in the Pre-NT sample is no longer present

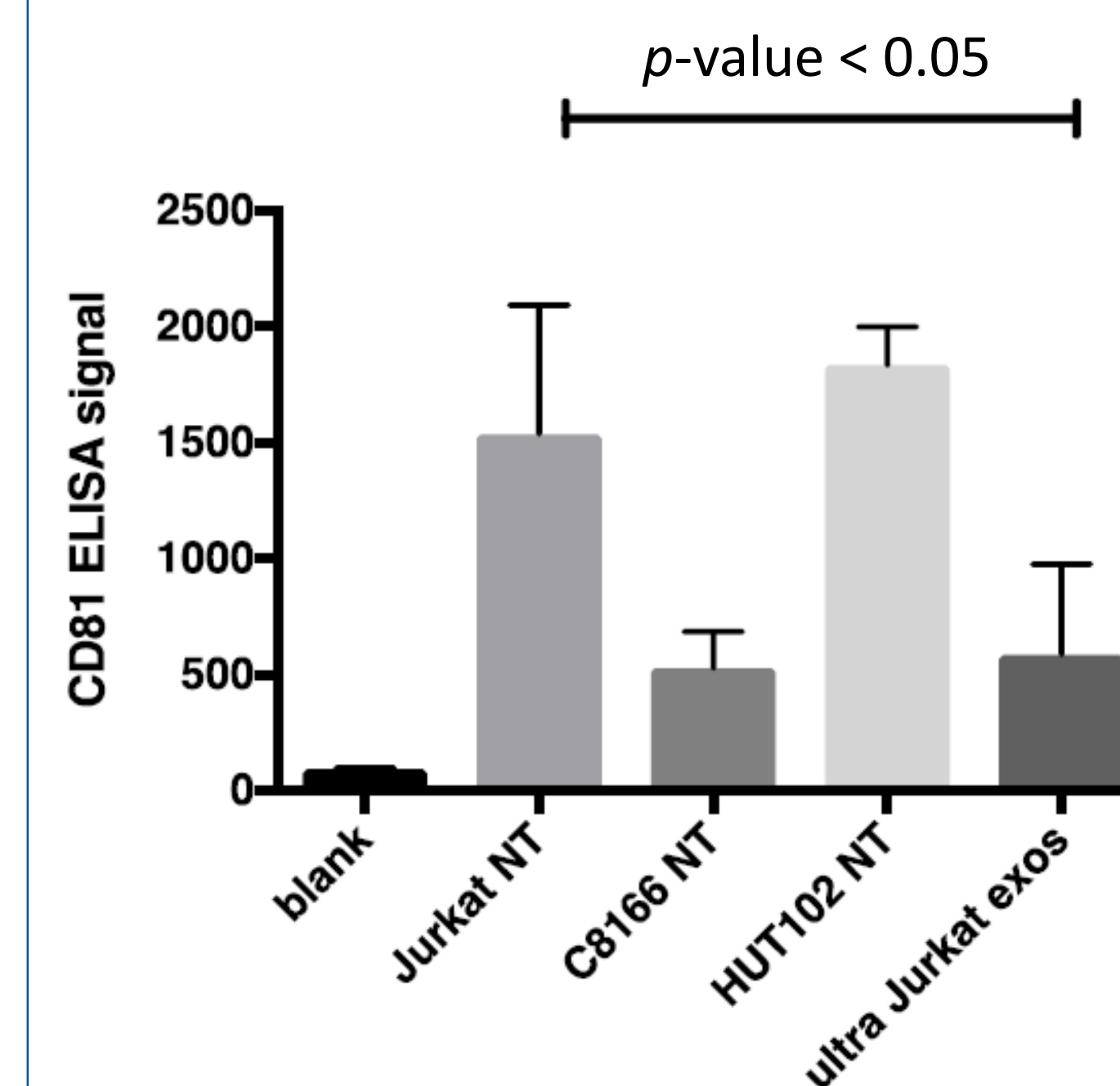


Nanotrap® particle sample processing is an efficient method for exosome isolation

Exosome enrichment: Nanotrap® Particles vs. Ultracentrifugation

Experimental design

- Exosomes were enriched from 10 mL of tissue culture media by ultracentrifugation (ultra Jurkat exos).
- Exosomes were enriched from 1 mL of tissue culture media using Nanotrap® particles (Jurkat NT, C8166 NT, and HUT102 NT).
- CD81 signals for exosomes were measured by electrochemical ELISA.

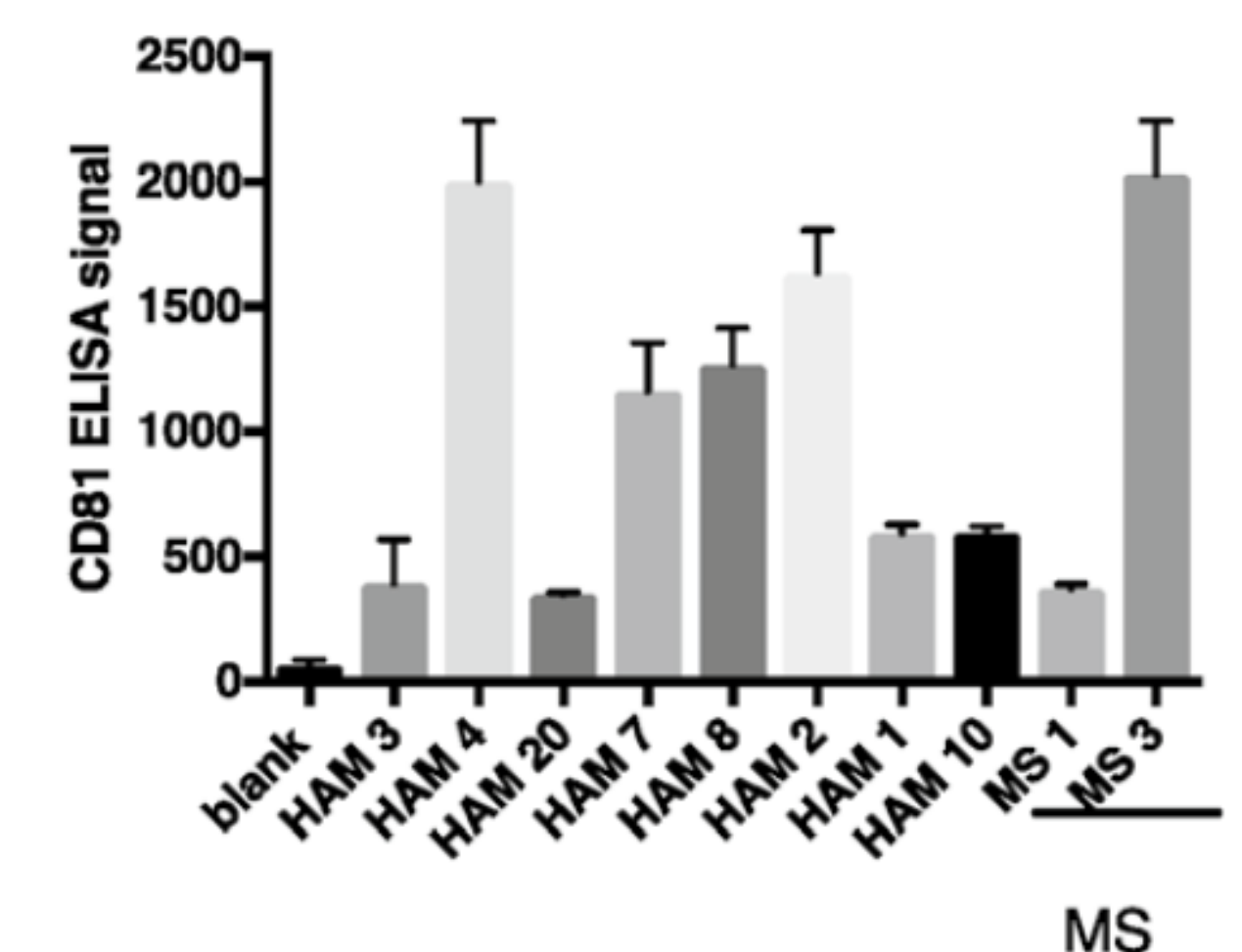


Nanotrap® particles can enrich exosomes to equal or **greater levels** than those isolated from **10 times** the same starting material by ultracentrifugation.

Nanotrap® Particles Enrich Exosomes from Cerebrospinal Fluid

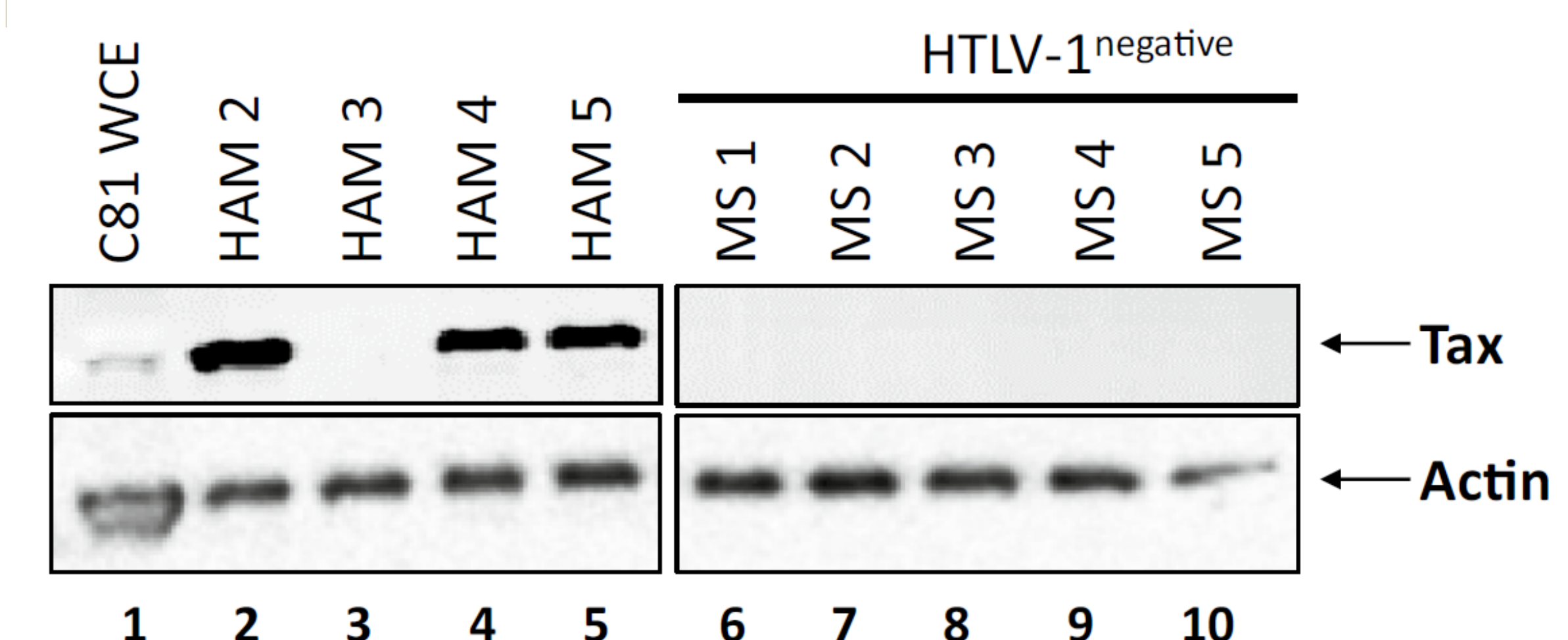
CD81 ELISA Activity

- Exosomes from cell-free CSF samples were enriched with Nanotrap® particles and analyzed for CD81 using an ELISA
- Nanotrap® particle enrichment enabled measurable exosome levels in all 8 HAM/TSP patient samples and in both of the Multiple Sclerosis (MS) patient samples



Western Blot Analysis

- Nanotrap® particle sample processing enriched exosomes with measurable levels of HTLV-1 Tax-protein in 3 of 4 initial HAM/TSP CSF samples
- Actin was measurable in all, confirming the presence of exosomes in both HAM/TSP and MS samples



Exosomes that contain viral proteins can be captured and enriched with Nanotrap® particles, allowing their evaluation as potential biomarkers