Jan 20, 2024

EVTrap non-antibody affinity bead protocol for EV isolation from urine, compatible with SiMOA assays

DOI
dx.doi.org/10.17504/protocols.io.81wgbxonylpk/v1

andrew.west west[1], yuan.yuan[1]

[1]Duke University

andrew.west west
Duke University

DOI: https://dx.doi.org/10.17504/protocols.io.81wgbxonylpk/v1

Protocol Citation: andrew.west west, yuan.yuan 2024. EVTrap non-antibody affinity bead protocol for EV isolation from urine, compatible with SiMOA assays. **protocols.io https://dx.doi.org/10.17504/protocols.io.81wgbxonylpk/v1**

Protocol status: Working
We use this protocol and it's working

Created: January 05, 2024

Last Modified: January 20, 2024

Protocol Integer ID: 92957

Keywords: ASAPCRN, evs from human urine, using evtrap bead, evtrap bead, evtrap, compatible with simoa assay, protocol for ev isolation, simoa assay, ev isolation, comparable elisa, human urine, detection of protein, ev, urine, using simoa, isolate

Funders Acknowledgements:
NIH/NINDS
Grant ID: NS064934
Michael J. Fox Foundation for Parkinson's Disease Research
Grant ID: MJFF-022434

Abstract

A modified protocol using EVTrap beads (Tymora Analytical) to isolate EVs from human urine, and subsequent processing, for detection of proteins using SiMOA or comparable ELISA-based protocols.

Materials

Reagents:

1. Tymora 10x Loading Buffer
2. Tymora Elution Buffer
3. Tymora EVTrap Beads

Troubleshooting

Preparation of Urine

1 Prechill the **AVANTI** centrifuge, add adapters (Prechilled).

Note

Do a pre-run with 10 of 15 mL conical tubes with water, to check for breakage or plastic warping), run for 00:20:00 at 1000 x g , 4 °C .

2 Thaw the urine in a shaking water bath at 42 °C 100 rpm . Leave the urine tubes On ice .

Note

Do not thaw at room temperature or in a refrigerated environment

3 Aliquot 250uL of urine into labeled 1.5mL tubes for creatinine measurements.

4 Transfer 5mL of urine to labeled 15mL conical tubes (keep tubes On ice)

5 Add ddH2O to each urine sample tube until volume reaches 7mL (keep On ice)

6 Centrifuge urine at 10000 x g for 00:10:00 at 4 °C to remove debris and large apoptotic bodies. 10m

7 Collect the cleared urine (keep On ice), leaving the pellet behind (keep On ice).

Capture of Extracellular Vesicles 4h 15m

8 Add the provided 10x Urine/Media Loading Buffer at 1-to-10 ratio to the urine sample (e.g. 700uL to 7mL urine).

9 Add 60uL Tymora EVtrap beads reagent.

10 Incubate for 04:00:00 by end-over-end rotation at 4 °C (make sure the beads freely move around). 4h

11 Spin samples at low speed, 200 x g (but, use 500x g if you don't see the right compact pellet) for 00:05:00. Check for compact pellet at bottom of tube. 5m

12 Prepare the washing buffer by diluting the 10x Urine/Media Loading Buffer at 1-to-10 ratio in PBS (e.g. 2mL buffer to 20mL PBS).

13 Remove supernatant and transfer to temporary tube.

14 Transfer beads to a labeled 1.5mL tube in 1mL of wash buffer (1x washing buffer) **by inverting the beads 10-20 times** and removing the wash solution.

15 Transfer supernatant back to original 15mL tube and save in -80 °C .

16 Isolate beads using magnetic separator and remove the wash solution.

17 Repeat wash step with cold PBS two more times, 1 mL each wash. Invert 10 times each.

18 Pulse spin (to get liquid out of cap).

19 Add 50 µL of the fresh Tymora Elution solution to the beads (the elution volume should be enough to fully resuspend the beads and allow for efficient interaction) and incubate beads 00:10:00 by vigorous shaking at 1000 rpm (eppemixer) at Room temperature . 10m

20 Place tube on a magnetic separator and collect elution buffer (~50ul) (this contains the EVs).

21 Repeat the elution step one more time with another 50uL of Tymora Elution solution and combine both elutions together. (~100uL)

22 Adjust pH to 7.4 using 1N HCl (make fresh, very carefully!) (7uL of acid per 100uL of elution solution).

23 Add 200 uL of Quanterix Homebrew sample diluent buffer (supplemented with 0.1% sodium dodecyl sulfate, 0.05% sodium deoxycholate, and 0.4 mM freshly prepared dithiothreitol).

24 If the sample is ice, sonicate sample 10sec at 10% power straight. >5 min wait on ice. 10sec at 10% power straight. (~20 sec of straight 10% probe-tip sonication).

25 Aliquot half of sample to a labeled 1.5mL Protein Low-Binding tube and store at -80 °C for future use.