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

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

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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



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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 \bigcirc 00:20:00 at \bigcirc 1000 x g , \bigcirc 4 °C .

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.

- Add ddH20 to each urine sample tube until volume reaches 7mL (keep 8 On ice)
- Centrifuge urine at 10000 x g for 00:10:00 at 4 °C to remove debris and large apoptotic bodies.
- 7 Collect the cleared urine (keep On ice), leaving the pellet behind (keep On ice).

Capture of Extracellular Vesicles

4h 15m

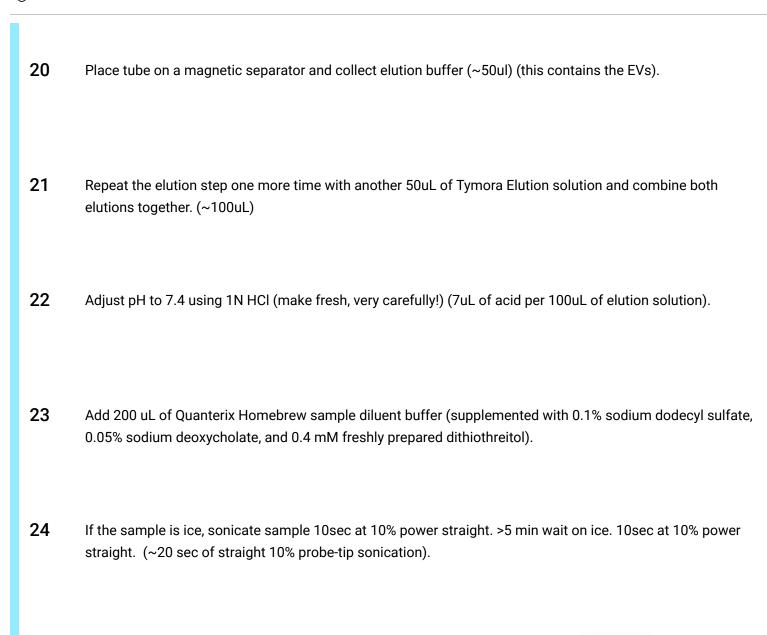
- Add the provided 10x Urine/Media Loading Buffer at 1-to-10 ratio to the urine sample (e.g. 700uL to 7mL urine).
- 9 Add 60uL Tymora EVtrap beads reagent.
- Incubate for 04:00:00 by end-over-end rotation at 4 °C (make sure the beads freely move around).
- Spin samples at low speed, 200 x g (but, use 500x g if you don't see the right compact pellet) for 00:05:00. Check for compact pellet at bottom of tube.

5m

4h

- 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).
- Remove supernatant and transfer to temporary tube.
- 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.
- Transfer supernatant back to original 15mL tube and save in 8 -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).
- 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 .

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Aliquot half of sample to a labeled 1.5mL Protein Low-Binding tube and store at

\$\circ\$ -80 °C for future use.