Exo-Urine™ EV Isolation Kit

Cat # EXOU100A-1

User Manual

Storage: Please see individual components

Version 1
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Contents

Product Description

Extracellular vesicles (EVs) released from cells have been an emerging and exciting field of research for basic and translational researchers. Through its circulation in biofluids such as urine, EVs play a role in mediating direct cell to cell communication, and provides new opportunities for researchers to study their contents for identification of novel biomarkers in both physiologically normal and disease states. Urinary-associated EVs (uEVs) have been a potential source of biomarkers for a host of kidney and genito-urinary disorders such as cancer (renal cell, prostate, and bladder), hypertension, and diabetic neuropathy – to name a few. As a non-invasive biofluid sample with respect to its collection, it has become a popular alternative to serum or plasma as a starting material for biomarker discovery.

However, urine as a biofluid poses technical challenges to researchers when used as a starting material for EV isolation. Due to high amounts of Tamm-Horstall Protein (THP) present in urine – a glycoprotein with a propensity for polymerization, EVs are typically trapped within its mesh network, and as a result EV yields and purity (irrespective of isolation techniques) have been generally poor and presents a challenge going forward for its widespread adoption.

To address the needs of researchers seeking to quickly isolate urinary-associated EVs at a purity and yield beyond what is commercially available, SBI is proud to introduce Exo-Urine™ EV Isolation Kit. Leveraging size-exclusion chromatography (SEC) with proprietary solubilization buffers, fractions with minimal THP/uromodulin and high EV-associated markers can be obtained with little as 1.5 ml of starting input in less than an hour. Additionally, the kit is designed for use with both freshly-voided (<4 hrs from initial collection) and frozen samples, providing maximal flexibility for the researcher on their choice of sample types.

List of Components

Item	Volume/Qty	Storage Temperature
Disposable empty gravity isolation column	5 columns	RT
Exo-Urine isolation resin	50 ml	4^0C
Equilibration buffer	100 ml	4^0C
Reagent A	375 µl	RT
Reagent B	375 µl	RT

Storage

The Kit is shipped on blue Ice and the components should be stored at recommended temperatures as stated above. Properly stored kits are stable for 12 months from the date received.

Protocol for Exo-Urine EV Isolation Kit:

1. Collect Urine and centrifuge at 200 x g for 10 minutes at 4°C to remove urinary cells and cell debris. Transfer supernatant (cell-free urine) to a new 15-50 ml conical tube (not provided). (**Note: Frozen urine (-20°C) should be thawed on ice on the day of exosome isolation. Fresh urine can be kept at 4°C for up to 4 hours for optimal results)**

2. Centrifuge the cell-free urine at 1800 x g for 10 minutes to remove any residual debris **(Note: Column preparation can be started at this point)**

3. Take 1.5 ml of the supernatant/cell-free urine and add it to a new tube (not provided).

Cell-free Urine is ready for exosome isolation. Keep it on ice till column preparation is completed.

Column preparation and EV capture

1. Take out the isolation column, place it on a rack or clamp it to a retort stand and cap the bottom closure with a red stopper.

2. Mix the Exo-Urine isolation resin by shaking the bottle thoroughly as contents may have settled at the bottom **(Note: For optimal results ensure that the resin is thoroughly mixed. The resin in the column shouldn't be allowed to dry out.)**

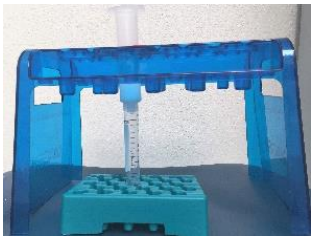
3. Pipette 10 ml of the Exo-Urine isolation resin onto the column. Insert and gently push the filter down with the help of a 15 ml conical tube (not provided) to pack the column uniformly (see picture). The filter should rest 1-2 mm above the resin bed. **(Note: avoid pushing the filter into the resin; tightly packed resin bed will restrict the column flow rate and efficiency.)**

4. Equilibrate by adding 10 ml of the equilibration buffer to column carefully by adding it along the wall of the column. Uncap the column by removing the red stopper and allow solution to completely flow through by gravity. Discard the flow through and cap the bottom again.

(Note: Both Exo-Urine isolation resin and equilibration buffer should be at the same temperature during the entire process (4°C or RT). Difference in temperature could affect the exosome isolation.)

Exosome isolation

1. Add 75 µl of Reagent A and 75 µl of Reagent B to the 1.5 ml cell-free Urine after column preparation is complete. Vortex to mix the sample.



2. Pipette the sample onto the column carefully.

3. Uncap the column and collect the eluate in a 15 ml conical tube (not provided). Allow the sample to completely flow through (see picture).

4. Add 10 ml of the equilibration buffer on to the column and continue to collect the eluate

5. Discard the first 8 ml of the total eluate (~2 ml flow-through from step #3, ~6 ml buffer from Step #4).

6. Collect and save the next 2 mls, which contains the desired EV fractions. The remaining fraction can be discarded or saved separately. **(Note: Do not let the resin dry out. For optimal results time between step 3 and 4 should be kept short).**

Example Data and Applications

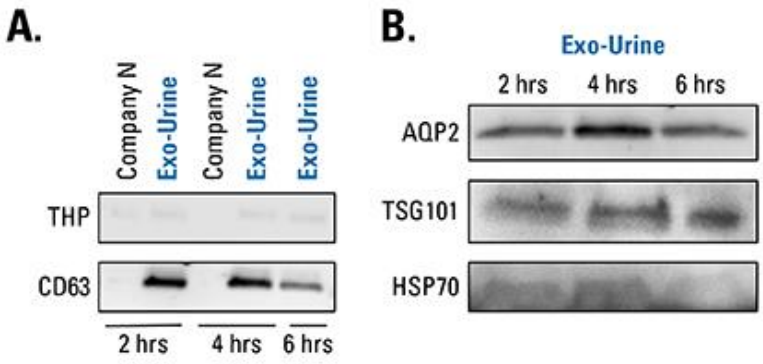


Figure 1. Urinary EVs (uEVs) were enriched using SBI's Exo-Urine and Company N's kit from freshly collected pooled urine samples from three healthy donors, and stored at the indicated time points (2 hrs, 4 hrs and 6 hrs) at +4^{0}C. The amount of urine used for Exo-Urine kit was 10-fold less (1.5 ml vs 15 ml) compared to Company N. Western blot analysis for (A) THP and CD63 comparing Exo-Urine and Company N's kit and (B) AQP2, TSG101, and HSP70 for Exo-Urine kit, demonstrating superior performance of Exo-Urine kit for delivering low level of contaminants and enrichment of EV-specific markers.

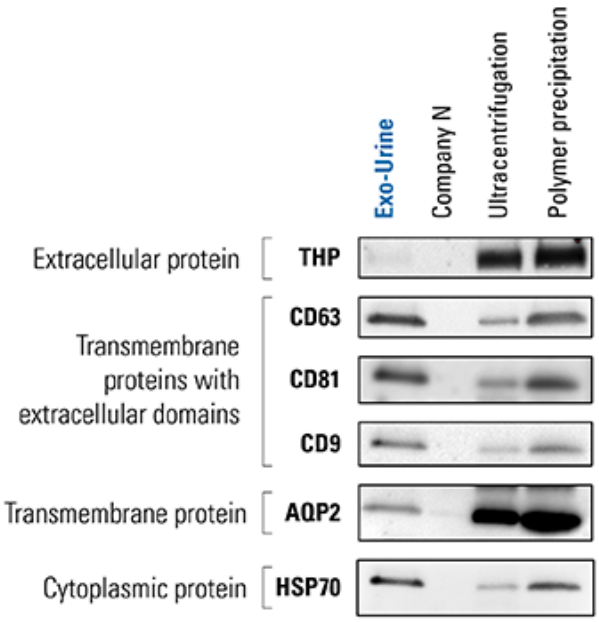


Figure 2. Urinary EVs (uEVs) were enriched from pooled urine sample stored at -200C for 3 weeks using Exo-Urine, Company N, UC and polymer precipitation isolation methods. The amount of urine used for Exo-Urine kit was 1.5 ml versus 15 ml of the same urine for the other methods. Different exosomal markers indicated above were probed to define the isolated uEVs from different methods (loaded based on equal volume ratio) using immunoblotting.

High relative amount of aquaporin-2 (AQP2) vs other EV markers (in UC/polymer precipitation lanes) indicates possible carryover of non-EV associated soluble form of aquaporin-2 (Kodaka et al. 2018)

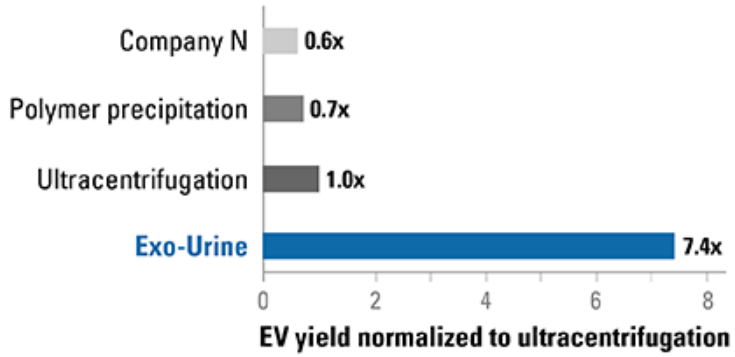


Figure 3. The Exo-Urine Kit delivers higher yields of EVs (protein equivalent) than other methods. EV preps from Figure 2 were analyzed for EV yield (as measured by protein equivalence using Qubit protein assay) and amounts normalized to the EVs isolated by ultracentrifugation. Use of the Exo-Urine Kit results in more EVs than all other methods.

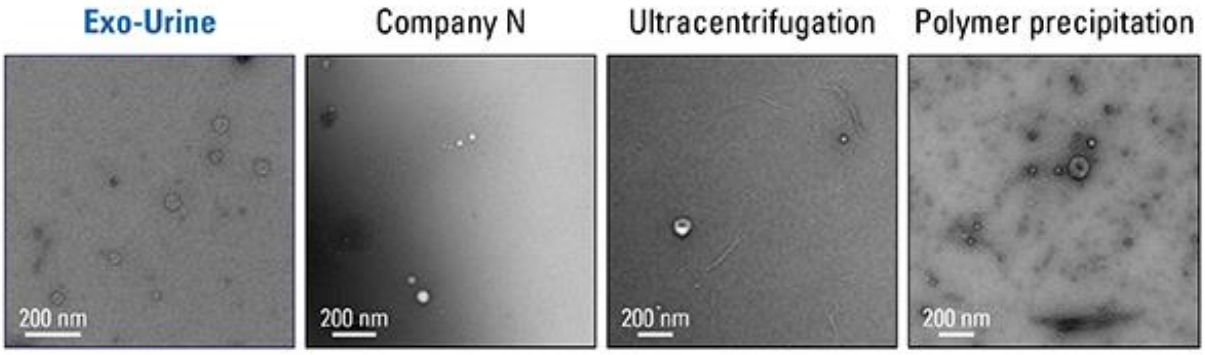


Figure 4. Transmission electron microscopy (TEM) of uEVs isolated by Exo-Urine, Company N, ultracentrifugation and polymer precipitation. Relative size and morphology of EVs are indicated. Note relative absence of THP filaments in Exo-Urine isolates compared to UC and polymer precipitation methods, showing increased purity of the Exo-Urine method

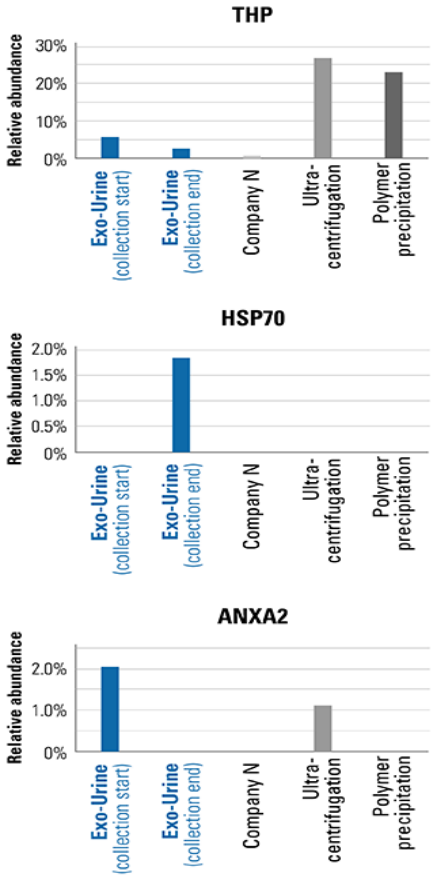


Figure 5. Mass spectrometry analysis of normalized protein abundance of THP, HSP70, and ANXA2 in Exo-Urine, Company N, UC and polymer precipitation isolates. The LC-MS/MS analysis demonstrates low relative abundance of THP and higher abundance of EV-associated markers HSP70 and ANXA2 in uEVs isolated by Exo-Urine kit.

References

1. Huebner AR et al. Exosomes in urine biomarker discovery. *Adv Exp Med Biol.* 2015; **845**:43-58. PMID: 25355568.

Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site: **http://www.systembio.com**

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