HansaBioMed Life Sciences

Pioneering the Extracellular Vesicles field

TFF-Flexi Beta Test Results

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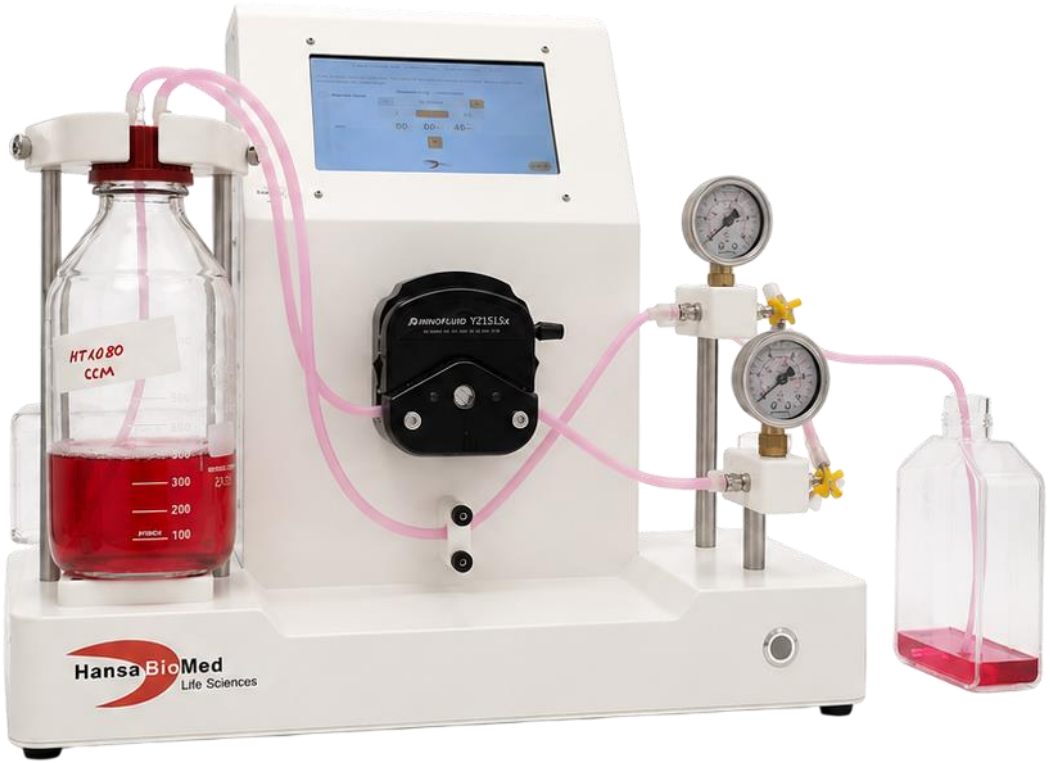
Cell Conditioned Medium - Workflow

Pioneering the EV field

- Used HT1080 cultured medium.
- First step is TFF-MV to remove large debris.
- Second step is TFF-EVs-S to get rid of <50nm protein contamination.
- Validated and characterized EVs through:
  - Size and concentration by NTA
  - Marker quantification by ELISA
  - Protein quantification by BCA
- 700mL of media processed in 1.5 hours total, including preparation time
- TFF-EVs-S retentate then purified further with PURE-EVs size exclusion chromatography columns, as per HBM's lyophilized EVs production workflow.

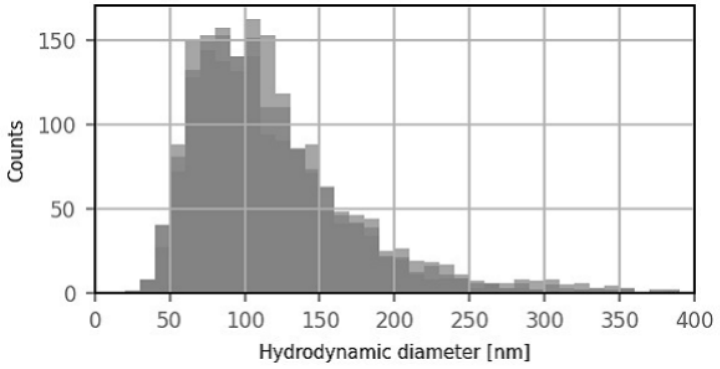


Cell Conditioned Medium - Results

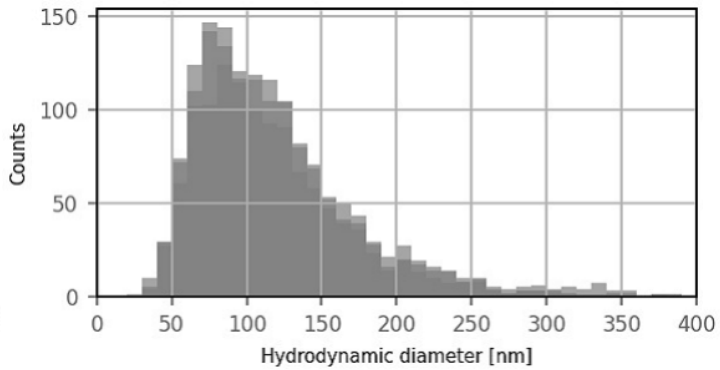
Pioneering the EV field

- Zetaview Evolution by Particle Metrix was used for size and concentration measurements.
- Expected size distribution profile from NTA:
  - Centered around 100nm peak,
  - Efficient removal of <50nm and >200nm particles.
- 86% total particle recovery before SEC.

Pre-SEC



Post-SEC

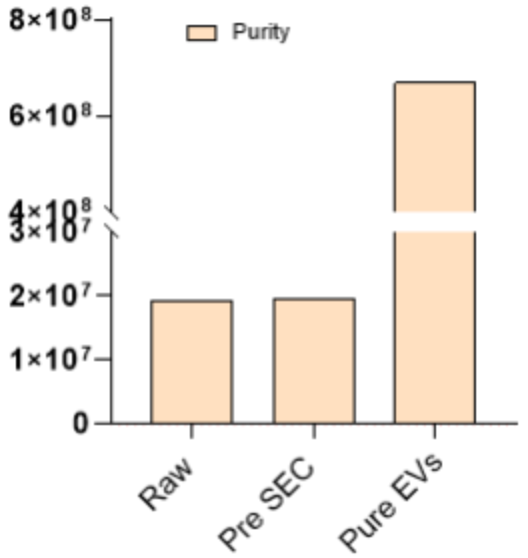


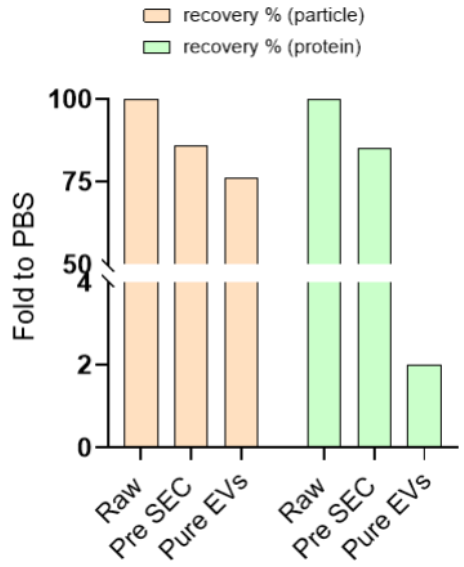
Sample name	Particle Conc (Part/ml)	Median X50 (nm)	Total Particle	Recovery part.
HT1080 Raw	1.07E+10	110.2	5.14E+12	100%
HT1080 TFF-EVs-S retentate	3.68E+11	106.0	4.42E+12	86%
HT1080 Pure EVs	2.61E+11	107.2	3.92E+12	76%

Cell Conditioned Medium - Results

Pioneering the EV field

- Protein quantification was performed with BCA.
- Particle count/protein quantity used as purity ratio [1].
- Efficient EV enrichment by TFF-MV→TFF-EVs→PURE-EVs workflow.





Sample name	Protein Conc. (ug/ml)	amount	Recovery protein	Purity
HT1080 Raw	560	480	100%	1.91E+07
HT1080 TFF-EVs-S retentate	18975	12	85%	1.94E+07
HT1080 PURE-EVs	391	15	2%	6.68E+08

[1] Webber J, Clayton A. How pure are your vesicles? J Extracell Vesicles. 2013 Jan 10;2. doi: 10.3402/jev.v2i0.19861. PMID: 24009896; PMCID: PMC3760653.

Cell Conditioned Medium - Results

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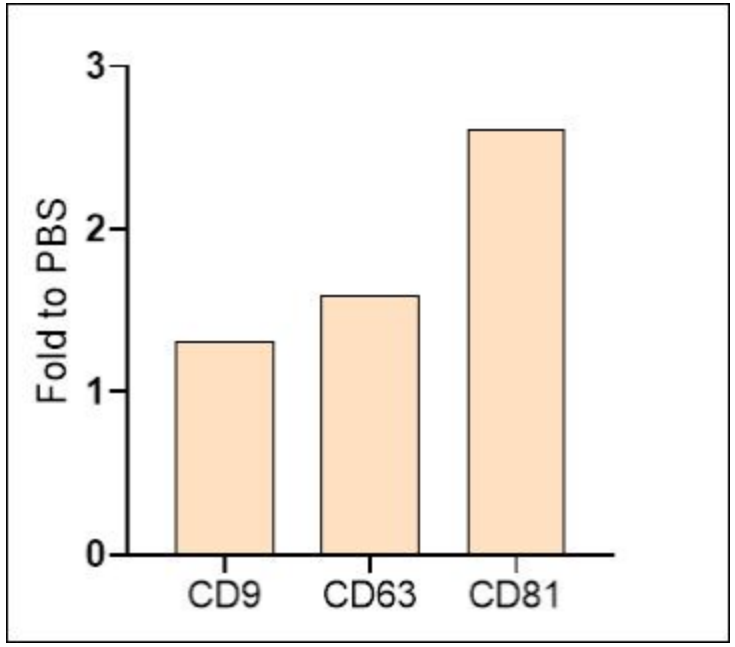
Pioneering the EV field

- Double-sandwich ELISA assays for surface marker assessment.
- CD9-coated plated used for EV capture.
- PBS used as backround (>1 values considered positive signals).
- Confirmed expression of EV markers.



Urine - Workflow

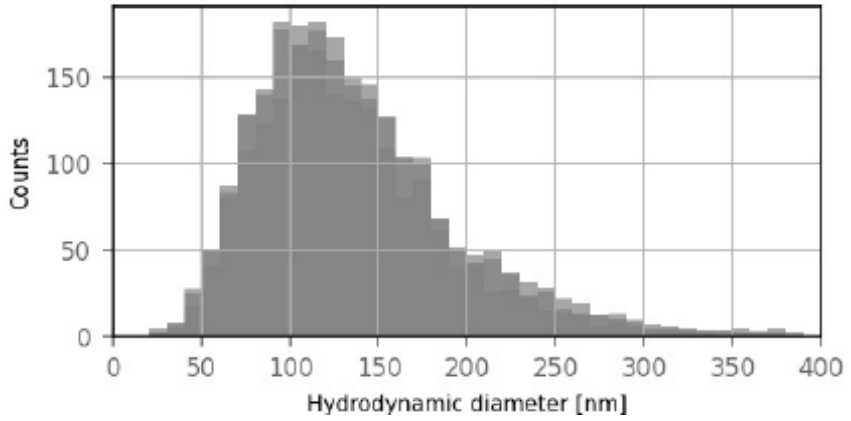
Pioneering the EV field

- Used 2 liters of urine as the starting material.
- First step is TFF-MV to remove large debris, but the large module (TFF-MV-L) is used due to large sample volume.
- Second step is TFF-EVs-L to get rid of <50nm protein contamination.
- Validated and characterized EVs through:
  - Size and concentration by NTA
  - Marker quantification by ELISA
  - Protein quantification by BCA
- 2L of media processed in 2.5 hours total, including preparation time
- TFF-EVs-S retentate then purified further with PURE-EVs size exclusion chromatography columns, as per HBM's lyophilized EVs production workflow.

Urine - Results

Pioneering the EV field

- Zetaview Evolution by Particle Metrix was used for size and concentration measurements.
- Expected size distribution profile from NTA:
  - Centered around 100nm peak,
- 58% total particle recovery after TFF-MV, efficient removal of >200nm particles.
- 2% total particle recovery in the TFF-EVs-filtrate (waste), efficient removal of <50nm particles.

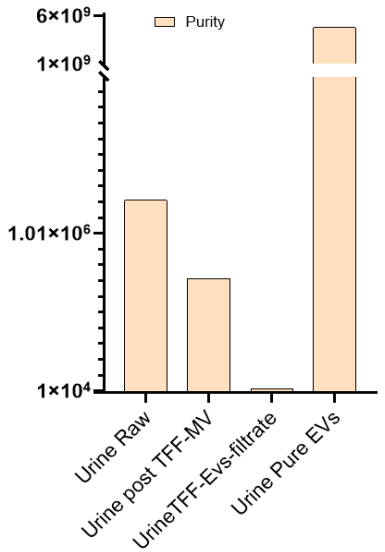


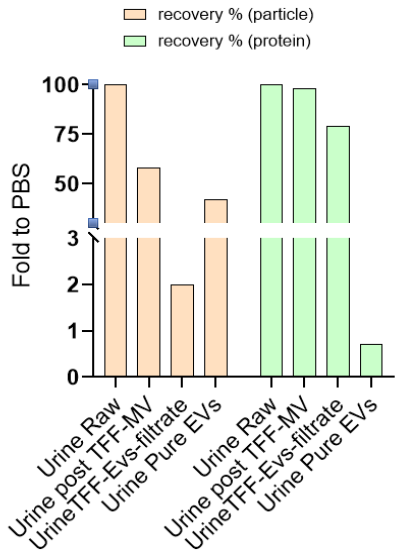
Sample name	Particle Conc (Part/ml)	Median X50 (nm)	Total Particle	Recovery part.
Urine Raw	1.52E+10	141.0	3.35E+13	100%
Urine post TFF-MV	8.87E+09	108.9	1.95E+13	58%
Urine TFF-EVs-filtrate	2.23E+08	112.5	5.35E+11	2%
Urine Pure EVs	4.25E+11	129.8	1.40E+13	42%

Urine - Results

Pioneering the EV field

- Protein quantification was performed with BCA.
- Particle count/protein quantity used as purity ratio [1].
- Efficient EV enrichment by TFF-MV→TFF-EVs→PURE-EVs workflow.



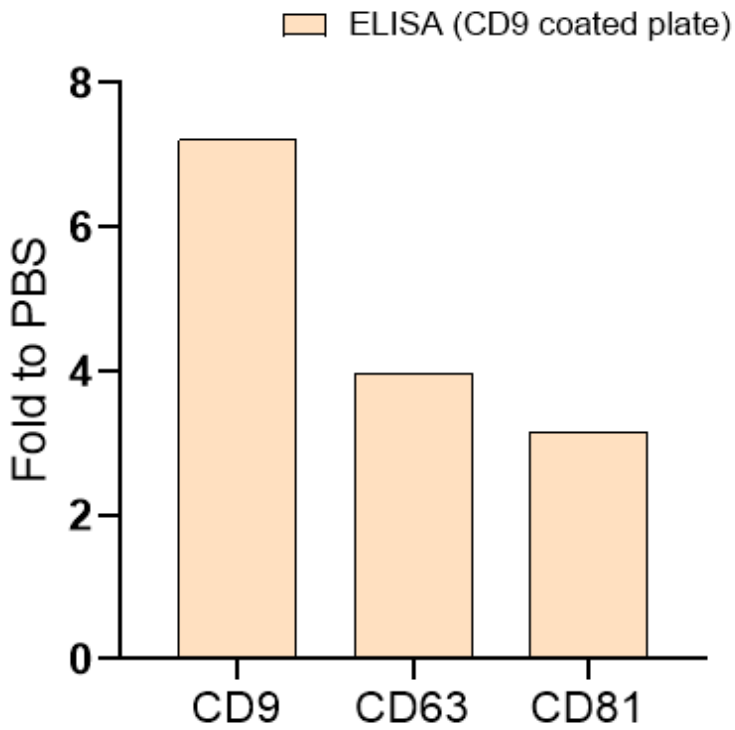


Sample name	Protein Conc. (ug/ml)	amount-ml	Recovery protein	Purity
Urine Raw	12466	2200	100%	1.22E+06
Urine post TFF-MV	12265	2200	98%	7.23E+05
Urine TFF-EVs-filtrate	9833	2400	79%	2.27E+04
Urine Pure EVs	89	33	0.7%	4.78E+09

Urine - Results

Pioneering the EV field

- Double-sandwich ELISA assay was performed on CD9-coated plate for quantification of surface markers.
- PBS is used as background (>1 implies positive signal).
- Presence of EV surface markers confirmed.



Conclusions

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- TFF-Flexi ensures high-throughput EV isolation.
- The automation still preserves the advantages of TFF cartridges in terms of yield and purity.