Exosomal mRNA Analysis kit from plasma and urine, "ExoComplete"

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1 Abstract

The ExoComplete kit is a seamless system capable of isolating exosomes and microvesicles (Extracellular Microvesicles: EMVs) from biological samples such as plasma and urine but also quantifying EMV mRNA. EMVs are membrane vesicles released from various cell types. They include exosomes (30 nm–150 nm in diameter) and microvesicles (100 nm–1000 nm in diameter). There is growing evidence that EMVs play a role in intercellular communication and their contents of proteins, lipids, miRNA, mRNA and DNA have clinical interest as potential biomarkers in diagnosis, prognosis and monitoring of various health conditions including cancer detection. Compared to conventional ultracentrifuge methods, our method can capture the equivalent or better yield of EMVs far more rapidly and high throughput. Accordingly, we launched the ExoComplete kit for research use in September 2015.

2 Characteristics of the New Product

- EVs can be captured easily in a short time
- Excellent reproducibility
- High-throughput

3 Background of the Development

Conventional methods such as ultracentrifugation, density gradient centrifugation and gel permeation chromatography are versatile and powerful methods for isolating EVs, these methods, however, have many issues such as reproducibility, complicated methodology, and lengthy protocols (procedures can take from 8 hrs to 30 hrs). In recent years, technical advances have been made in the field of EV isolation. Improving knowledge and emerging novel technologies include magnetic beads and column methods using antibodies or lectin for quick and easy detection. These methods, however, still demonstrate low reproducibility and difficulty in processing many samples. Here, we have developed a seamless and high-throughput method to capture EVs from clinical samples (urine, plasma, etc.) and to purify mRNA in a few steps. We have used a previously developed mRNA purification platform and combined it with an exosome isolation technology. **Figure 1** shows the procedural flow. This kit can accommodate a range of sample volumes and has two types of flow. The tube format (up to 12.5 mL) can be used for large volume specimens such as urine, cell culture media, etc. and the 96-well plate format (up to 0.4 mL) can be used for small volume specimens such as plasma and serum, etc.

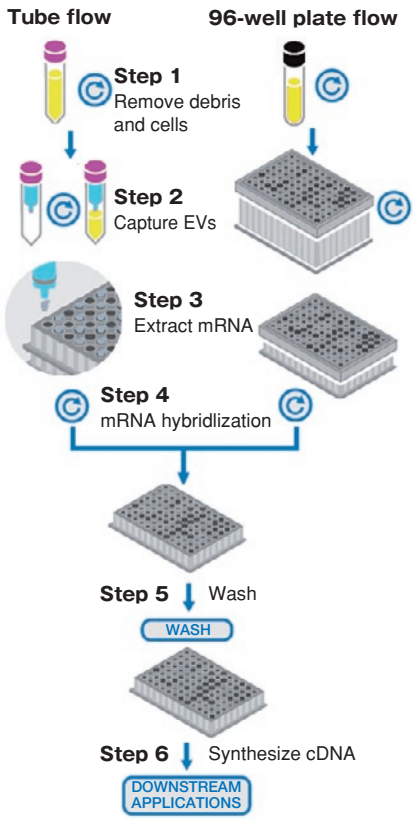

Figure 1 Procedural flow

4 Technical Details

In order to isolate exosomes, and micro vesicles (30 nm–150 nm), we examined a filter material base with a combination of electrostatic and size-exclusion properties. Furthermore, using two-layer membranes with different pore sizes reduced clogging and improved EVs capture.

1) Verification of captured EVs

Scanning electron microscope (SEM) analysis of urinary particles captured on the filter fiber. The exosomes were labeled with anti-CD63 antibody (exosome surface marker) and colloidal gold with silver enhancement. The filter was analyzed by the backscattering mode (**Figure 2**).

2) Comparison with conventional methods (ultracentrifugation)

EVs were captured from the same urine sample (10 mL) by the ExoComplete kit or conventional ultracentrifugation method followed by gene expression analysis of the housekeeping genes, β- actin (ACTB) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) by qPCR. The data confirmed that ExoComplete provides comparable or even superior results to the ultracentrifugation method in terms of mRNA assay sensitivity and reproducibility (**Figure 3**).

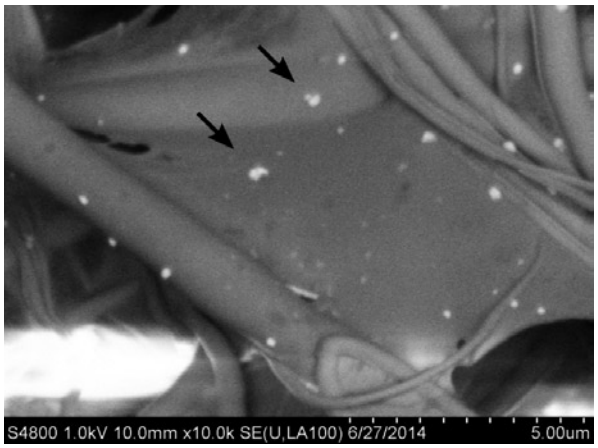

Figure 2 SEM image of EVs captured in the filter membrane [1)]

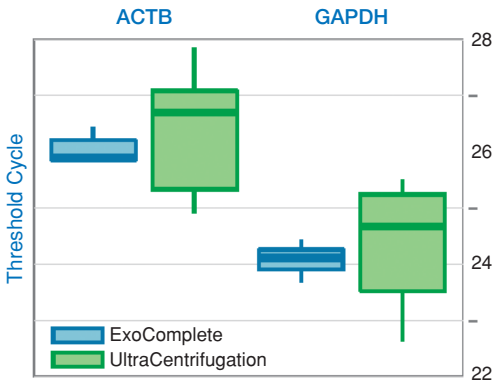

Figure 3 Comparing the conventional method (ultracentrifugation) to our method

5 Future Business Development

・Deploy to clinical inspections in inspection centers

【Relevant patents】

US Patent Nos. 5976797, 6638428, 6844158
7258976, 7214781/ 7374881/7741023/7745180/7939300, 7981608, 7968288, 8076105, 8101344, 7816081, 7838239/ 8268982, 8268566; JP4772055, EP1802776, ZL200580035896.6, JP4945554, KR10-0983450, JP5706913

【Reference】

1) Murakami T, et al. Development of Glomerulus-, Tubule-, and collecting Duct-Specific mRNA Assay in Human Urinary Exosomes and Microvesicles. PLOS/One 2014: Vol 9 Issue 10 e109074