Ver4.3

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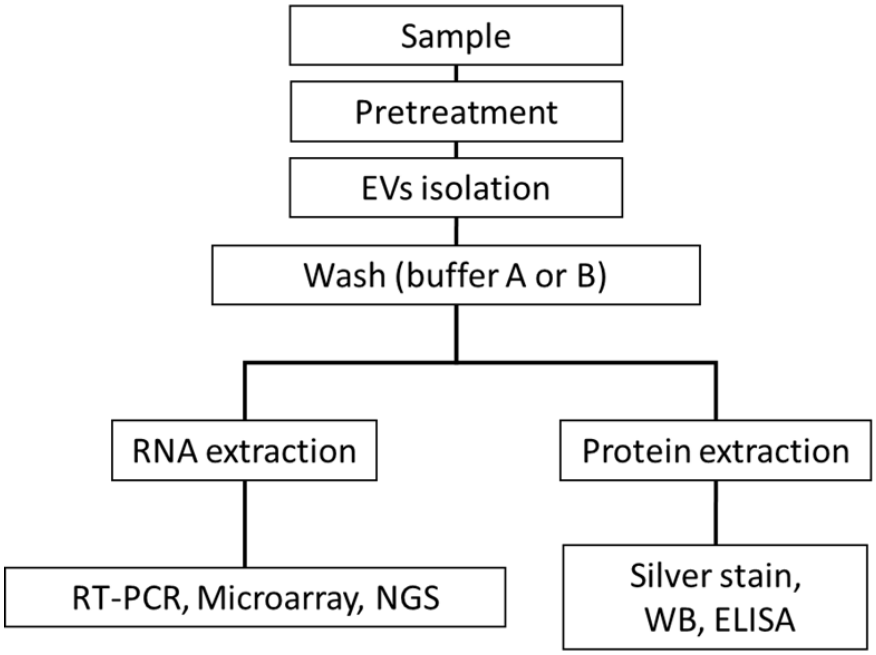
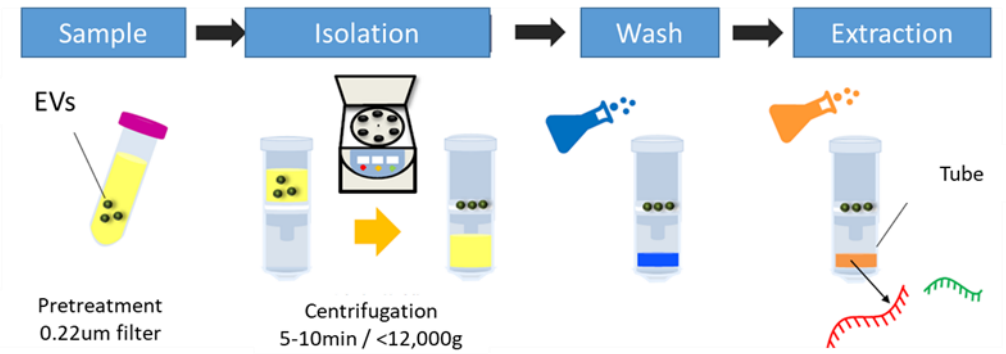
EVAGLAX™ usage protocol

（Applicable product：SC-B-730-00-00）

1. Overview of how to use EVAGLAX™





2. Kit contents

Goods	20 assays	100 assays
Spincolumn	20 pcs	100 pcs
Collection tube (1.5mL)	20 psc	100pcs
Wash buffer A	10mL	50mL
Wash buffer B	10mL	50mL

3. Reagents other than this kit (not supplied)

Category	Name	Product	Manufacture	Cat No.
Common	0.2μm Pre-filter	Minisart Syringe Filter 0.2μm	Sartorius	S6534FMOSK
Common	1mL Syringe	-	-	-
Common	1.5mL low protein adsorption tube	-	-	-
Common	2.0mL low protein adsorption tube	-	-	-
For RNA	miRNeasy	miRNeasy Micro Kit	QIAGEN	217084
For Protein	M-PER	Mammalian Protein Extraction Reagent	Thermo Fisher Scientific	78501

1. Storage

Store the kit in a refrigerated environment at 2-10°C.

2. Purpose of use

This product is for research use only. Please do not use this product for medical or clinical diagnosis of humans or animals.

3. EVs purification procedure

【Sample preparation process】

・If the specimen has been frozen, ensure it is thawed at 4° C.

・Apply a 0.2 μm pre-filter

*If the specimen contains a lot of debris or is highly viscous, it is recommended to perform pre-centrifugation at 10,000 G/30 min first and then filter the supernatant with a pre-filter. Take care to retain 150 μL of supernatant after pre-centrifugation.

【Exosome capture process】

・Add 100-500 μL of the sample to the spin column.

・Centrifuge at 6,000G/10min. (10,000G/3min, if insufficient)

*If clogging is a concern, set the temperature of the centrifuge machine to 25°C.

・Discard the tube containing the flow-through and insert the spin column to a new 1.5 mL low-protein adsorption tube.

(*If there are many contaminants in the sample, select and add the following operation.)

(If specimen contaminants in the sample is slightly high)

・Add100 μL of Wash buffer A to the spin column and centrifuge at 3,000 G/ 5 min.

・Discard the tube containing the flow-through and insert the spin column to a new 2.0 mL low-protein adsorption tube.

(If there are many contaminants in the specimen)

・Add 300 μL of Wash Buffer B to the spin column and centrifuge at 3,000 G/5 min.

・Discard the tube containing the flow-through and insert the spin column to a new 2.0 mL low-protein adsorption tube.

4. EVs lysis procedure

【Protein extraction process】

・Add 100uL of M-PER to the spin column and centrifuge at 500G/1min.

*Do not discard the flow-through. Use the 2.0 mL low-protein adsorption tube without changing it.

・Keep at room temperature for 5 minutes.

・Centrifuge at 6,000G/5min. (10,000G/3min, if you need)

・The obtained flow-through is the sample.

【RNA extraction process】

・Add 500uL of QIAzol to the spin column and centrifuge at 500G/1min.

*Do not discard the flow-through. Use the 2.0 mL low-protein adsorption tube without changing it.

・Keep at room temperature for 5 minutes.

・Centrifuge at 6,000G/5min. (10,000G/3min, if insufficient)

・ Transfer the obtained flow-through to a new 1.5 mL low-protein adsorption tube. No spin column is required.

・Add 140uL of chloroform, stir with Vortex for 15 seconds and keep at room temperature for 3 minutes.

・Separate into 3 layers by centrifuging at 12,000 G at 4° C for 15 minutes. Since the top transparent layer is a water layer containing RNA, Aspirate 280 μL of the transparent layer only so as not to absorb the white and red layers below, and transfer to another 1.5 mL tube.

・Add 1.5 volumes of 100% EtOH (420 uL in this case) and stir by pipetting.

-----The following is according to the miRNeasy protocol (numbers are modified) ----- ・

・Transfer 700uL (as much as possible) of sample to the RNeasy MinElute spin column, cap gently, and centrifuge at 8,000G for 15 seconds. Discard the flow-through.

(*If necessary for NGS, perform DNase treatment at this moment. Use RNase-Free DNase Set (QIAGEN).

Add 350uL of Buffer RWT ⇒ 8,000G, 15min

⇒Add DNase and react for 10 min

⇒ Add 350uL of Buffer RWT ⇒ 8,000G, 15min

Note that if performing this operation, washing with 700uL of BufferRWT is unnecessary.)

・Add 700uL of Buffer RWT to the spin column, cap gently, and centrifuge at 8,000G for 15 seconds. Discard the flow-through.

・Add 500uL of Buffer RPE to the spin column, cap gently, and centrifuge at 8,000G for 15 seconds. Discard the flow-through.

・Add 500uL of 80% EtOH to the spin column, cap slowly, and centrifuge at 8,000G for 2min. Discard the collection tube containing the flow-through.

・Insert the RNeasy MinElute spin column into a new collection tube (2 mL in the kit). Centrifuge at maximum speed (>12,000G) for 5 min with the column lid open to dry.

・Insert the RNeasy MinElute spin column into a new collection tube (1.5 mL in the kit). Add 14uL of RNase-free water (small container) to the spin column (Do not stick to the walls! Keep the pipette tip away from the filter!), cap slowly, and centrifuge at maximum speed (>12,000G) for 1 min to extract RNA.
(*The liquid that comes out is the sample)

Document Revision History

Date	Ver	Changes
June 2020	1.3	Create new
September 2020	1.4	Updated text
October 2020	1.5	Updated text
October 2020	1.6	Updated text
February 2021	1.7	Updated text
March 2021	1.8	Updated text
June 2021	2.0	Updated text
July 2021	3.0	Updated text
January 2022	4.0	Updated text EVAGLAX™
February 2022	4.1	Updated Fig.
March 2022	4.2	Updated text
November	4.3	Updated text

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