CODE No. MEX-SA

For Research Use Only. Not for use in diagnostic procedures.

Printed July 5, 2017
Version 2.0

ExoCap™ Streptavidin Kit

Exosome Isolation and Enrichment Kit

PRODUCT DESCRIPTION

ExoCap™ Streptavidin Kit is designed for the customized isolation and analysis of exosomes or microvesicles, called "Extracellular Vesicles (EVs)", using the researcher's biotinylated molecules such as antibodies against exosome surface marker proteins, of which representatives are tetraspanins such as CD9 and CD63. Exosomes are extracellular vesicles secreted by most cell types and contain various marker proteins and RNAs, such as microRNA and fragmented mRNA. ExoCap™ Streptavidin Kit uses functionalized Magnosphere™ magnetic microparticles for exosome isolation. These beads are coated with a JSR Life Sciences proprietary hydrophilic polymer to decrease non-specific binding.

EXAMPLE APPLICATIONS

ExoCap™ Streptavidin Kit allows for exosome isolation from serum, plasma and cell culture supernatant. Isolated exosomes can be used for western blotting, flow cytometry, beads CLEIA and qRT-PCR.

PRODUCT COMPONENTS

ExoCap™ Components	Feature	Amount
Streptavidin Magnetic Beads*	Beads diameter: 3 µm Solid content: 0.2% (2 mg/mL) Number of particles: 1×10^8 beads/mL approx.	2 mL
Treatment Buffer	Optimized assay diluent for analyte	30 mL
Washing/Dilution Buffer	Effect: keeping dispersibility of the beads	60 mL

*__Note__: **Streptavidin Magnetic Beads** have a binding capacity to biotin-labeled antibody. The binding capacity of beads is approximately 3 µg IgG per mg Streptavidin Magnetic Beads.

TEST NUMBERS BY DOWNSTREAM APPLICATION

Downstream Application	Test numbers
Western blotting	20 tests
Flow Cytometry	Maximum 160 tests
CLEIA	Maximum 160 tests
qRT-PCR	8 tests

STORAGE

Store at 2-8°C, DO NOT FREEZE.

REQUIRED MATERIALS

- Biotinylated antibody
  Commercially available biotin-labeled antibodies or in-house made biotinylated antibodies can be used†.
  † Commercially available reagent for biotinylation of antibody is EZ-Link NHS-LC-Biotin (Cat. # 21336), manufactured by Thermo Scientific, has been confirmed to work with ExoCap™ Streptavidin Kit.
- Biotinylated protein (if desired)
- Magnet stand (Magnetic rack)
  Tube stand for 1.5 and/or 2 mL tube
- Magnet plate (for CLEIA)
  Plate-type magnet for 96 well assay plate
- Vortex mixer and Sample shaker
- PBS
  Buffer for suspending bead-exosome complex
- Casein Sodium (CAS No. 9005-46-3)
  Sample preparation for qRT-PCR

PROTOCOL

Beads Preparation

1: Preparation of Streptavidin Magnetic Beads with biotinylated antibody (Capture Beads)

Streptavidin Magnetic Beads (0.2%)	Washing/Dilution Buffer for re-suspending	Biotinylated antibody	Final re-suspending volume (0.2%)	Test size		
				WB	FCM or CLEIA	qRT-PCR
0.5 mL	1.0 mL	5 μg	0.5 mL	5 tests	40 tests	2 tests
1.0 mL	1.0 mL	10 μg	1.0 mL	10 tests	80 tests	4 tests
1.5 mL	1.0 mL	15 μg	1.5 mL	15 tests	120 tests	6 tests
2.0 mL	1.0 mL	20 μg	2.0 mL	20 tests	160 tests	8 tests

1) Suspend **Streptavidin Magnetic Beads** by vortex, then transfer the required volume of the beads slurry into a 2 mL tube according to the above table.
2) Collect the beads by placing the tube on magnetic stand for about 1 minute and discard the supernatant carefully.
3) Re-suspend the beads with 1 mL of **Washing/Dilution Buffer**.
4) Add recommended volume* of **biotinylated antibody** into the tube (see above table).
   ***Note**: The ratio of antibody to magnetic beads is an important parameter to maximize the signal-to-noise ratio. It is recommended to apply excess amount of antibody for each assay because ligand-free binding site of streptavidin could cause the increase of background noise, due to undesirable non-specific adsorption. For instance, it is shown that a kind of alkaline phosphatase has some non-specific binding/adsorption to ligand free streptavidin beads.
5) Rotate the tube for 30-60 minutes at room temperature or 2-8°C.
6) Collect the beads by magnetic separation and discard the supernatant.
7) Wash the beads 3 times with 0.5 mL of **Washing/Dilution Buffer**.
8) Re-suspend the beads with **Washing/Dilution Buffer** with reference to the above table.
9) Antibody - Streptavidin beads (**Capture Beads**) can be stored at 2-8°C. The storage period depends on the original antibody stability.

Sample Preparation

2: Cell and Debris Pre-Clearance Procedure for samples

1) Prepare the appropriate size tube for your sample (serum, plasma or cell cultured supernatant).
2) Dispense the sample into the tube.

3) Centrifuge the tube at 300 × *g* at 4°C for 10 minutes.
4) Transfer the supernatant to a new tube and discard cell pellet.
5) Centrifuge the tube at 2,000 × *g* at 4°C for 20 minutes.
6) Transfer the supernatant to a new tube and discard dead cell pellet.
7) Centrifuge the tube at 10,000 × *g* at 4°C for 30 minutes.
8) Transfer the supernatant to a new tube and discard cell debris pellet.
9) Option: Filter the final supernatant with a 0.22 μm filter unit.
10) The sample is now ready for immediate use with **ExoCap™ Streptavidin Kit** or stored at -80°C until just before use, if desired.

Exosome isolation

[Important Notice-A]
Add equal volumes of **Treatment Buffer** to sample volume to minimize non-specific binding of exosome on the beads.

- Example:

  Serum/Plasma 100 μL + Treatment Buffer 100 μL = Total volume 200 μL
  Serum/Plasma 500 μL + Treatment Buffer 500 μL = Total volume 1,000 μL

A preliminary reaction condition for exosome capture is 2-8°C, 18-24 hours.

Note: Please modify the conditions in accordance with the purpose, if desired, since maximum reaction rate and quality of result depend on both time and temperature (see below).

(a) Optimize for high exosome concentration conditions by reducing reaction time or sample volume.
(b) Optimize for low exosome concentration conditions by incubating at room temperature, increasing sample volume, or increasing beads amount (except for Flow Cytometry).

[Important Notice-B]
In case of use of body fluid such as Serum and Plasma, the issue of **HAMA**, Human anti-mouse antibody, might exist in the experiment of Flow Cytometry and CLEIA. In that case, 0.01% Mouse IgG addition could improve HAMA issue.

- Example:

  Treatment Buffer 1 mL + 1% Mouse IgG solution 10 μL = 0.01% Mouse IgG in Treatment Buffer

If you have HAMA issue in your sample, please try to use the above buffer in your flow cytometry and CLEIA experiment.

[Important Notice-C]
In case of sample preparation for qRT-PCR, it is recommended to use Treatment Buffer with 1% Casein to avoid non-specific binding of non-exosomal nucleic acid.

- Example:

  Transfer 50 mg of Casein Sodium (CAS No. 9005-46-3) to a polypropylene tube. Add 5 mL of Treatment Buffer and stir the solution for a few hours.

  Note: The casein solution should be stored in the freezer if you do not use it immediately.

◆ Western blotting Preparation (Protocol 3 and 4)

3: Exosome Capture Procedure for Western blotting

- Example : 100 μL* beads, 100 μL sample

  * Recommended **Capture Beads** amount for **Western blotting** sample preparation. You may titrate the Capture Beads amount according to your target abundance.

1) Set 2 mL tubes on a magnetic tube stand.
2) Vortex to suspend **Capture Beads**, prepared in *"Protocol 1: Preparation of Streptavidin Magnetic Beads with biotinylated antibody"*, and transfer 100 μL of the beads slurry into each tube per sample.
3) Place the tube on the magnetic tube stand for about a minute and remove the supernatant.
4) Add 100 μL (or equivalent sample volume) of **Treatment Buffer** and suspend the beads well.
5) Add 100 μL of **sample** that has been cleared of cells and debris.

Note: Please see above *"Protocol 2; Cell and Debris Pre-Clearance Procedure"*.

6) Incubate the sample for 18-24 hours at 2-8°C or room temperature with gentle mixing.
   Note: Optimize capture conditions by titrating sample volume, time, and temperature of reaction conditions. Please refer to the [**Important Notice-A**].
7) Briefly spin the tube to remove beads from the top of the tube.
8) Place the tube on the magnetic tube stand for about a minute and remove the supernatant.
9) Wash the beads 2 times with 500 µL **Washing/Dilution Buffer**. Mix the beads briefly but thoroughly. After 2 times washing, re-suspend with 500 µL **Washing/Dilution Buffer** and transfer the contents to a fresh tube.
10) Place the tube on the magnetic tube stand for about a minute and remove the supernatant carefully.
11) Option: In order to elute the exosome proteins for Western blotting, you may add the appropriate lysis buffer directly to the beads according to your preferred method/kit. In that case, place the tube on the magnetic tube stand for about a minute and collect the eluted sample.

4: Preparation Protocol for SDS-PAGE

Continued from Protocol **3: Exosome Capture Procedure for Western blotting**,

12) Add 20 µL of 1× SDS Sample Buffer (Laemmli's sample buffer) directly to the beads and mix well.
13) Incubate the sample for 10 minutes at room temperature with gentle mixing.
14) Place the tube on magnetic tube stand about a minute and transfer supernatant to a fresh tube.
    Note: If reducing conditions are desired, add reduction agent.
15) Incubate 95°C for 5 minutes.
16) Vortex and spin down.
17) Apply the sample to a lane of SDS-polyacrylamide gel for electrophoresis.
18) Load the samples onto SDS-PAGE, and proceed to Western blotting analysis.

◆ Flow Cytometry Preparation (Protocol 5 and 6)

5: Exosome Capture Procedure for Flow Cytometry

➢ Example : 12.5 µL* beads, 50 µL sample
  * This protocol is adjusted for both microcentrifuge tube and 96 well plate.

1) Set 2 mL microfuge tubes or 96 well plate on magnet stands.
2) Suspend **Capture Beads** (prepared in *"Protocol 1: Preparation of Streptavidin Magnetic Beads with biotinylated antibody"*) thoroughly using a vortexer and transfer 12.5 µL of the suspension into each tube or plate well.
3) Place the tube or plate on the magnet for about a minute and remove the supernatant.
4) Add 50 µL of **Treatment Buffer** and suspend the beads well.
   Note: If prevention of **HAMA** in serum or plasma is required, please refer to the [**Important Notice-B**].
5) Add 50 µL of **sample** that has been cleared of cells and debris.
   Note: Please see above *"Protocol 2; Cell and Debris Pre-Clearance Procedure"*.
6) Incubate the sample for 18-24 hours at 2-8°C or room temperature with gentle mixing.
   Note: Optimize capture conditions by titrating sample volume, time, and temperature of reaction conditions. Please refer to the [**Important Notice-A**].
7) Briefly spin the tube to remove beads from the top of the tube, if a tube is used.
8) Place the tube or 96 well plate on the magnet for about a minute and remove the supernatant.
9) Wash the beads 2 times with 100 µL **Washing/Dilution Buffer**.
10) Re-suspend beads in 250 µL of PBS.
    Note: 250 µL beads in PBS will allow for 8 replicates (25 µL/well) for flow cytometry analysis.

6: Preparation of exosome-bead complexes for Flow Cytometry

Continued from **5: Exosome Capture Procedure for Flow Cytometry**,

11) Dispense 25 µL of the beads slurry into a 96 well plate or tube.
12) Add optimized volume of fluorescent antibody against target of interest or isotype control.
    Note 1: Please refer to your antibody instruction.

Note 2: Fluorescent antibodies from MBL (MEDICAL & BIOLOGICAL LABORATORIES. CO., LTD.) are recommended.

13) Add PBS and adjust the total volume to 100 μL.
14) Mix gently by rotator for 1 hour at room temperature.
15) Place 96 well plate on the magnet for about a minute and remove the supernatant.
16) Wash the beads 3 times with 100 μL PBS.
17) Add 250 μL of PBS.
18) Perform flow cytometry analysis with maximum counts at 100,000 beads / sample.

◆ CLEIA Preparation (Protocol 7)

7: Exosome Capture Procedure for CLEIA

➢ Example : 12.5 μL* beads, 50 μL sample

* Recommended **Capture Beads** amount for **CLEIA** sample preparation.

1) Set 96 well plate on magnet.
2) Suspend **Capture Beads** (prepared in *"Protocol 1: Preparation of Streptavidin Magnetic Beads with biotinylated antibody"*) thoroughly using a vortexer and put 12.5 μL of the suspension into each well in the 96 well plate.
3) Place the 96 well plate on the magnet for about a minute and remove the supernatant.
4) Add 50 μL of **Treatment Buffer** and suspend the beads well.
   Note: If prevention of **HAMA** in serum or plasma is required, please refer to the [**Important Notice-B**].
5) Add 50 μL of **sample** that has been cleared of cells and debris.
   Note: Please see above Protocol 2; Cell and Debris Pre-Clearance Procedure.
6) Incubate the sample for 20 minutes to 24 hours at 2-8°C or room temperature with gentle mixing.
   Note: Optimize capture conditions by titrating sample volume, time, and temperature of reaction conditions. Please refer to the [**Important Notice-A**].
7) Place 96 well plate on the magnet for about a minute and remove the supernatant.
8) Wash the beads 3 times with 100 μL of **Washing/Dilution Buffer**.
9) Remove the supernatant on the magnet.
10) Add the exosome related **secondary antibody** conjugated with alkaline phosphatase (ALP)
11) Incubate the beads and antibody for 20 minutes at room temperature.
12) Wash the beads at least 3 times with 100 μL of TBS/0.1% Tween 20.
13) Add the optimal substrate‡ to the beads, and incubate for appropriate time (about 5 to 30 minutes) at room temperature to develop the reaction.
    ‡**Note**: Thermo Scientific, ELISA-Light™ Immunoassay System with CDP-Star® and Emerald-II™ Substrate/Enhancer Solution, Cat.#T1026, has been verified with **ExoCap™ Streptavidin Kit**.
14) After the optimal reaction time, measure the chemiluminescence signal by using a microplate reader.

◆ qRT-PCR Preparation (Protocol 8 and 9)

8: Exosome Capture Procedure for qRT-PCR

➢ Example : 250 μL* beads, 300 μL sample

* Recommended **Capture Beads** amount for **qRT-PCR** sample preparation. You may titrate the Capture Beads amount according to your target abundance.

1) Set 2 mL tubes on a magnetic tube stand.
2) Vortex to suspend **Capture Beads**, (prepared in *"Protocol 1: Preparation of Streptavidin Magnetic Beads with biotinylated antibody"*), and transfer 250 μL of the beads slurry into each tube per sample.
3) Place the tube on the magnetic tube stand for about a minute and remove the supernatant.
4) Add 300 μL (or equivalent sample volume) of **Treatment Buffer with 1% Casein** and suspend the beads well.
   Note: Please refer to the [**Important Notice-C**].
5) Add 300 μL of **sample** that has been cleared of cells and debris.
   Note 1: Please see above *"Protocol 2; Cell and Debris Pre-Clearance Procedure"*.

Note 2: Adjust the dilution rate and the volume with **Washing/Dilution Buffer** as needed.

6) Incubate the sample for 18-24 hours at 2-8°C or room temperature with gentle mixing.
   Note: Optimize capture conditions by titrating sample volume, time, and temperature of reaction conditions. Please refer to the [**Important Notice-A**].
7) Briefly spin the tube to remove beads from the top of the tube.
8) Place the tube on the magnetic tube stand for about a minute and remove the supernatant.
9) Wash the beads 1 time with 1,000 µL **Washing/Dilution Buffer**. Mix the beads briefly but thoroughly. Transfer the contents to a fresh tube.
10) Place the tube on the magnetic tube stand for about a minute and remove the supernatant carefully.
11) Wash the beads 2 times with 1,000 µL **Washing/Dilution Buffer**. Mix the beads briefly but thoroughly.

9: Preparation Protocol for qRT-PCR

Continued from **8: Exosome Capture Procedure for qRT-PCR**

12) Place the tube on the magnetic tube stand for about a minute and remove the supernatant carefully.
13) Re-suspend with **Washing/Dilution Buffer** as required for nucleic acid isolation.
    Note 1: Please refer to your instruction of nucleic acid extraction and PCR.
    Note 2: **ExoCap™ Nucleic Acid Elution Buffer** from MBL (MEDICAL & BIOLOGICAL LABORATORIES. CO., LTD.) is recommended.

Related Products

Code	Name	Clone	Volume/Package	Product Type	Isotype	Application	Cross reactivity
MEX001-3	Anti-CD9 mAb	A100-4	100 µg/100 µL	non-labeled mAb	Mouse IgG2a	WB, IP, FCM	Hu, Mky
MEX002-3	Anti-CD63 (LAMP-3) mAb	C047-1	100 µg/100 µL	non-labeled mAb	Mouse IgG2b	WB, IP, FCM	Hu, Mky
MEX003-3	Anti-CD81 (TAPA1) mAb	A103-10	100 µg/100 µL	non-labeled mAb	Mouse IgG2a	WB, IP, FCM	Hu, Ham, Mky
MEX001-6	Anti-CD9 mAb-Biotin	A100-4	50 µg/50 µL	Biotihylated mAb	Mouse IgG2a	WB, FCM	Hu
MEX002-6	Anti-CD63 (LAMP-3) mAb-Biotin	C047-1	50 µg/50 µL	Biotihylated mAb	Mouse IgG2b	WB, FCM	Hu
MEX003-6	Anti-CD81 (TAPA1) mAb-Biotin	A103-10	50 µg/50 µL	Biotihylated mAb	Mouse IgG2a	WB, FCM	Hu
MEX004-6	Anti-CD326 (EpCAM) mAb-Biotin	B8-4	50 µg/50 µL	Biotihylated mAb	Mouse IgG1	FCM	Hu
M075-6	Mouse IgG1 (isotype control)-Biotin	2E12	50 µg/50 µL	Biotihylated mAb	-	FCM	-
M076-6	Mouse IgG2a (isotype control)-Biotin	6H3	50 µg/50 µL	Biotihylated mAb	-	FCM	-
M077-6	Mouse IgG2b (isotype control)-Biotin	3D12	50 µg/50 µL	Biotihylated mAb	-	FCM	-
M078-6	Mouse IgG3 (isotype control)-Biotin	6A3	50 µg/50 µL	Biotihylated mAb	-	-	-
MEX1001	ExoDiluent for Immunoassay	-	50 mL	-	-	-	-
MEX-E	ExoCap™ Nucleic Acid Elution Buffer	-	20 assays	-	-	-	-
3190	Magnetic Rack	-	1 unit (1.5 mL x 8 tubes)	-	-	-	-

Other related products are also available.
Please visit our website at http://ruo.mbl.co.jp/. You can check the updated information on website.

Manufacturer

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.

URL http://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

IMPORTANT NOTICE AND TERMS AND CONDITIONS

For Research Use Only. Not for use in diagnostic procedures.

This product is for research use only and shall not be used for human or animal diagnostic, therapeutic or commercial purposes. You shall not use this product to manufacture commercial products without prior notification to and approval by MEDICAL & BIOLOGICAL LABORATORIES CO., LTD. or any of its affiliates. (MEDICAL & BIOLOGICAL LABORATORIES CO., LTD. and its affiliates are collectively or severally referred to as "MBL".)

This product must be used by or directly under the supervision of a technically qualified person as defined by the federal Toxic Substances Control Act (or, if this product is used outside U.S.A. and if an equivalent law or regulation exists in the place of use, such law or regulation), who can understand the risks of exposure associated with the product. See 40 C.F.R. Part 720.

MBL shall not be held responsible or liable for infringement of any patent or any other intellectual property rights of any third parties that may occur with the use of this product.

This product is not for resale. This product may not be transferred or resold to third parties, modified for resale, or used to provide a service to third parties without written approval of MBL.

Any unused materials must be disposed of according to local regulations.

Failure to comply with these terms and conditions may subject you to claims for breach of contract and/or other penalties or damages under applicable laws or regulations.

The specificity of this product may change without notice.

MBL does not guarantee that this product will be continuously available.

MBL makes no warranties as to this product including, but not limited to, implied warranties of merchantability or fitness for a particular purpose. By use of this product you shall be deemed to have accepted these terms and conditions**.**

"ExoCap™" is a trademark of JSR Corporation.