Human CD9 x CD63 Expressed Exosome Detection ELISA Kit

Catalog # 6038

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA Kit to quantify human exosomes expressing CD9 and/or CD63
FORMAT:	Pre-coated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	5 hours
STANDARD RANGE:	250 ng/ml to 4 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Culture Media, Serum, Plasma, and Purified Samples
RECOMMENDED SAMPLE DILUTIONS:	1:1 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	N/A
NOTES:	

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Chondrex, Inc. provides a human CD9 X CD63 expressed exosome quantitative ELISA kit for cell culture media, serum, plasma, and purified samples.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard - Human CD9 X CD63 Peptide Conjugate (60381)	1 vial	250 ng, Lyophilized	-20°C
Detection Antibody (60383)	1 vial	100 µl	-20°C
Solution B - Sample/Standard/Detection Antibody Dilution Buffer (60364)	1 bottle	50 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer (9055)	1 bottle	20 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 µl	-20°C
TMB (90023)	2 vials	0.2 ml	-20°C
Chromogen Dilution Buffer (90022)	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016)	1 bottle	10 ml	-20°C
Wash Buffer, 20X (9005)	1 bottle	50 ml	-20°C
Capture Antibody Coated 96-Well ELISA Plate (Green)	1 each	8-well strips x 12	-20°C

NOTES BEFORE USING ASSAY

NOTE 1: It is recommended that the standard and samples be run in duplicate.

NOTE 2: Warm up all buffers to room temperature before use.

NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20°C for use in a future assay.

NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

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ASSAY OUTLINE

Add 100 µl of Solution B into wells

↓ Incubate at room temperature for 1 hour.
Wash plate.

Add 100 µl of diluted standards and samples into wells

↓ Incubate at room temperature for 2 hours.
Wash plate.

Add 100 µl of diluted Detection Antibody Solution into wells

↓ Incubate at room temperature for 1 hour.
Wash plate.

Add 100 µl of diluted Streptavidin Peroxidase Solution into wells

↓ Incubate at room temperature for 30 minutes.
Wash plate.

Add 100 µl of diluted TMB Solution into wells

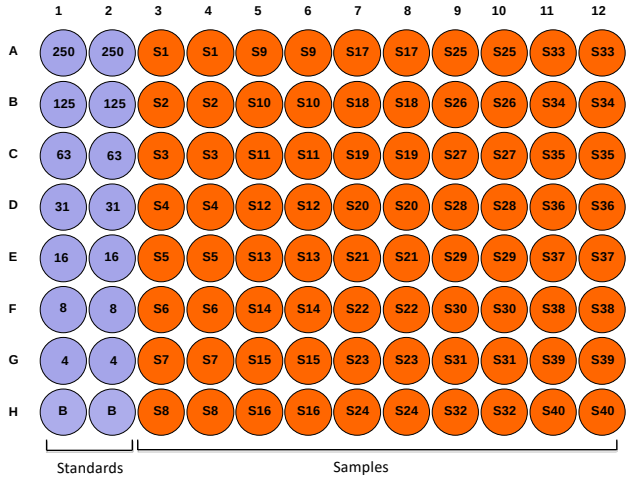
↓ Incubate at room temperature for 25 minutes.

Add 50 µl of Stop Solution into wells

↓

Read plates at 450 nm/630 nm

PLATE MAPPING



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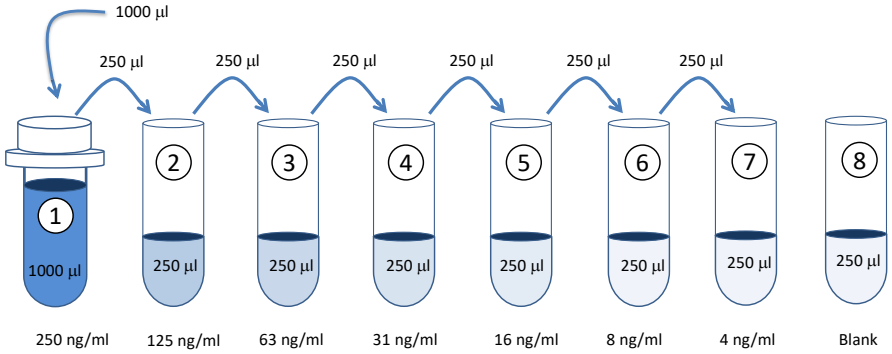
ASSAY PROCEDURE

1. **Add Blocking Buffer**: Add 100 µl of the Sample/Standard/Detection Antibody Dilution Buffer (Solution B) to each well and incubate for 1 hour at room temperature.

2. **Prepare Standard Dilutions**: The recommended standard range is 4 - 250 ng/ml. Dissolve one vial of human CD9 X CD63 peptide conjugate standard in 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B) for the 250 ng/ml standard. Then serially dilute it with Solution B. For example, mix 250 µl of the standard (250 ng/ml) with an equal volume of Solution B to make a 125 ng/ml solution, and then repeat it five more times for 63, 31, 16, 8, and 4 ng/ml solutions. The remaining 250 ng/ml standard stock may be stored at -20°C for use in a second assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



3. **Prepare Samples:** Exosomes can be stored at 4°C for up to 1 week. For long-term storage, keep at -20°C or -80°C. If multiple assays are required, Chondrex, Inc. recommends aliquoting samples into tubes which will only undergo one freeze/thaw cycle in order to avoid damaging the exosomes.

   Cell Culture Media: Remove cell debris and insoluble precipitate by centrifuging at 10,000 rpm for 5 minutes. When not in use, store the supernatant samples at -20°C; avoid repeat freeze-thaw cycles.

   Serum*: Clot blood samples by incubating samples at room temperature for 2 hours. Collect serum by centrifuging samples at 10,000 rpm for 5 minutes. When not in use, store the serum supernatant samples at -20°C; avoid repeat freeze-thaw cycles.

   Plasma*: Collect plasma samples with the use of anticoagulants such as heparin. Collect plasma by centrifuging samples at 10,000 rpm for 5 minutes within 30 minutes of blood collection. When not in use, store the plasma supernatant samples at -20°C; avoid repeat freeze-thaw cycles.

   NOTE: Using lipemic (cloudy) samples may affect assay results. The stored samples must be centrifuged at 10,000 rpm for an additional 5 minutes before the assay.

   Dilution: Dilute samples at least 1:1 with Solution B depending on the estimated exosome levels in the samples. Two to three different sample dilutions are recommended if the exosome levels in the samples are unknown.

   NOTE: Samples must be diluted with Solution B to maintain optimal assay conditions.

4. **Dilute Wash Buffer**: Dilute 50 ml of Wash Buffer, 20X in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

5. **Add Standards and Samples**: Add 100 µl of Solution B (blank), standards, and samples to designated wells in duplicate and incubate at room temperature for 2 hours.

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6. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Detection Antibody Solution**: Dilute one vial of detection antibody with 10 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B). Add 100 µl of detection antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	Detection Antibody (µl)	Solution B (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

8. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

9. **Add Streptavidin Peroxidase Solution**: Dilute one vial of streptavidin peroxidase solution with 10 ml of Streptavidin Peroxidase Dilution Buffer (Solution D). Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 30 minutes.

Strip #	Streptavidin Peroxidase (µl)	Solution D (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

10. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

11. **Add TMB Solution**: Dilute one vial of TMB in 10 ml of Chromogen Dilution Buffer just prior to use. Add 100 µl of TMB solution to all wells immediately after washing the plate and incubate for 25 minutes at room temperature.

Strip #	TMB (µl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

12. **Stop**: Stop the reaction with 50 µl of 2N Sulfuric Acid (Stop Solution) to each well.

13. **Read Plate**: Read the OD values at 450 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

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CALCULATING RESULTS

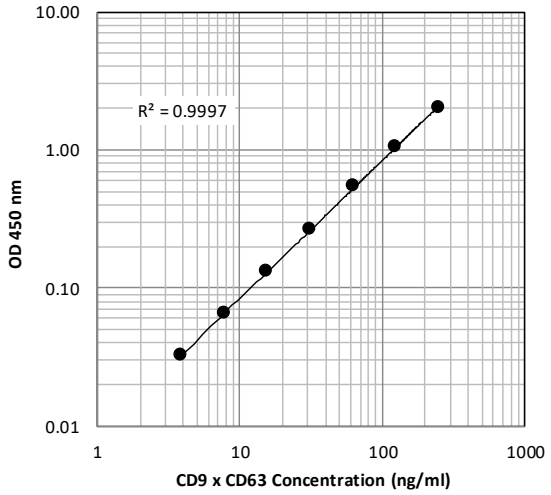
1. Average the duplicate OD values for the blank, standards, and test samples.

2. Subtract the “blank” (B) values from the averaged OD values in step 1.

3. Plot the OD values of standards against the concentration of CD9 X CD63 peptide conjugate (ng/ml). Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is 4 - 250 ng/ml.

4. The ng/ml of CD9 X CD63 expressed exosomes in test samples can be calculated using regression analysis.

Figure 1 - A Typical Standard Curve for the Human CD9 X CD63 Expressed Exosome Detection ELISA Kit



TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.’s ELISA FAQ for more information.

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