Chondrex, Inc.

Human CD9 Expressed Exosome Detection Kit

Catalog # 6036

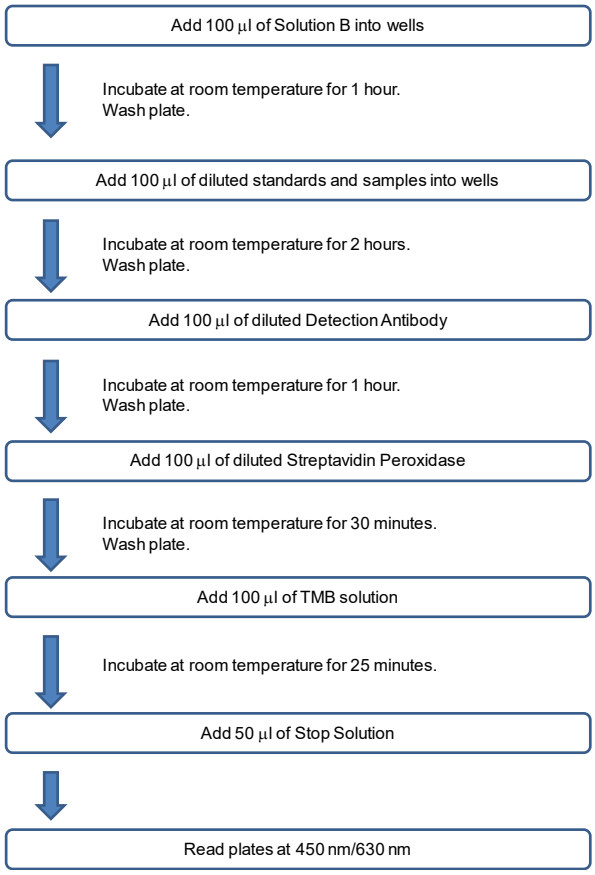
For Research Use Only - Not Human or Therapeutic Use

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

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard - Human CD9 Peptide Conjugate (60361)	1 vial	50 µg/ml, 0.1 ml	-20°C
Detection Antibody (60363)	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 (Yellow)	1 each	8-well strips x 12	-20°C

ASSAY OUTLINE



2607 151st Place NE
Redmond, WA 98052

Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094

www.chondrex.com
support@chondrex.com

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: Partially used reagents may be kept at –20°C.

Note 4: 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 dissolved completely.

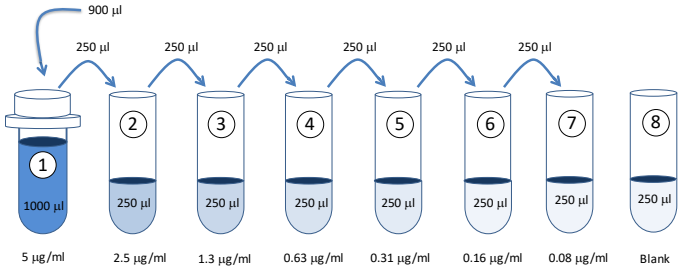
Note 5: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

Note 6: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

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 0.08 - 5 µg/ml. Dilute one vial (0.1 ml) of human CD9 peptide conjugate standard with 0.9 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B) for the 5 µg/ml standard. Then serially dilute it with Solution B. For example, mix 250 µl of the standard (5 µg/ml) with an equal volume of Solution B to make a 2.5 µg/ml solution, and then repeat it five more times for 1.25, 0.63, 0.31, 0.16, and 0.08 µg/ml solutions. The remaining 5 µg/ml standard stock may be stored at -20°C for use in a second assay. We recommend 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, we recommend aliquotting 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.

   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.

   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.

   *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 CD9 expressed exosome level in the samples. Two to three different sample dilutions are recommended if the CD9 expressed exosome levels in the samples are unknown.

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

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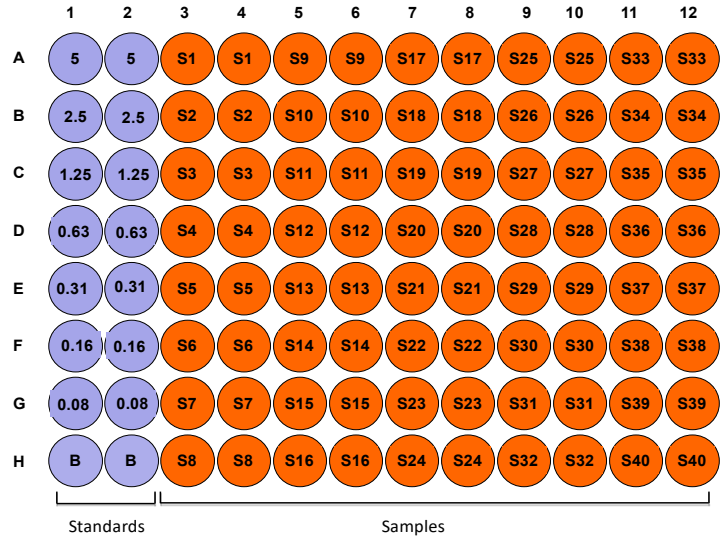
4. **Dilute Wash Buffer:** Dilute 50 ml of Wash Buffer, 20X in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *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 according to the layout in Figure 1. Incubate at room temperature for 2 hours.

Figure 1 - A Standard Assay Layout



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 blot 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 in 10 ml of solution B. Add 100 µl of detection antibody solution to each well and incubate at room temperature for 1 hour.

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 blot 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 in 10 ml Streptavidin Peroxidase Dilution Buffer (Solution D). Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 30 minutes.

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 blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

11. **Add TMB Solution**: Use new tubes when preparing TMB solution. Just prior to use, dilute one vial of TMB solution with with 10 ml Chromogen Dilution Buffer. Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature.

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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CALCULATION OF 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 peptide conjugate (μg/ml). Using a log/log plot will linearize the data. Figure 2 shows a representative experiment where the standard range is 0.08 - 5 μg/ml.
4. The μg/ml of CD9 expressed exosomes in test samples can be calculated using regression analysis.

Figure 2 - A typical standard curve for CD9 expressed exosome ELISA

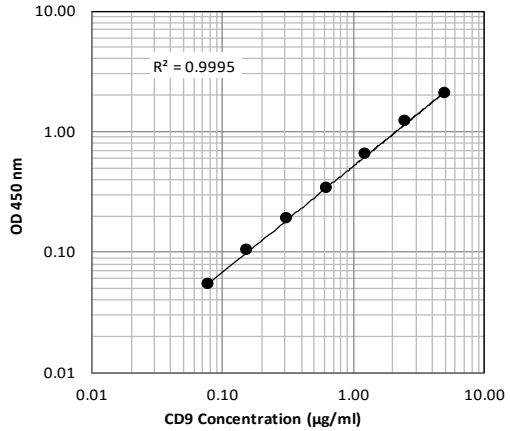

Table 1 - Reproducibility for CD9 ELISA Kit

Test	0.31 μg/ml	1.25 μg/ml	5 μg/ml
Inter-Assay CV (%)	4.7	9.2	3.0
Intra-Assay CV (%)	4.1	4.0	7.6
Spike Test*	79%	89%	88%

* Known amounts of CD9 peptide was added to normal mouse serum and then diluted with Sample/Standard/Detection Antibody Dilution Buffer (Solution B).

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