# Rat Tumor Necrosis Factor Alpha Detection ELISA Kit

Catalog # 6901

*For Research Use Only - Not Human or Therapeutic Use*

## PRODUCT SPECIFICATIONS

| | |
|---|---|
| DESCRIPTION: | ELISA Kit to quantify Rat TNF-α |
| FORMAT: | Pre-coated 96-well ELISA Plate with removeable strips |
| ASSAY TYPE: | Sandwich ELISA |
| ASSAY TIME: | 4 hours |
| STANDARD RANGE: | 5000 - 78 pg/ml |
| NUMBER OF SAMPLES: | Up to 40 (duplicate) samples/plate |
| SAMPLE TYPES: | Culture Media, Serum, and Plasma |
| RECOMMENDED SAMPLE DILUTIONS: | 1:1 (at least) |
| CHROMOGEN: | TMB (read at 450 nm) |
| STORAGE: | -20°C for 12 months |
| VALIDATION DATA: | Intra-Assay (1.1-21%)/Inter-Assay (12.7-20.7%)/Spiking Test (88-94%) |
| NOTES: | |



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Chondrex, Inc. provides a rat tumor necrosis factor alpha (TNF-α) quantitative ELISA kit for cell culture, serum, and plasma. samples.

## KIT COMPONENTS

| Item | Quantity | Amount | Storage |
|---|---|---|---|
| Rat TNF-α Standard (69011) | 2 vials | 5000 pg, lyophilized | -20°C |
| Detection Antibody (69013) | 2 vials | 50 µl | -20°C |
| Solution B - Sample/Standard/Detection Antibody Dilution Buffer (67015) | 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 | 200 µl | -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 (Blue) | 1 each | 8-well Strips x12 | -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 -20ºC 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 diluted standards and samples into wells

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

Add 100 µl of diluted Detection Antibody

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

Add 100 µl of diluted Streptavidin Peroxidase

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

Add 100 µl of TMB solution

↓ Incubate at room temperature for 25 minutes.

Add 50 µl of Stop Solution

↓

Read plates at 450 nm/630 nm

## PLATE MAPPING

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | 5000 | 5000 | S1 | S1 | S9 | S9 | S17 | S17 | S25 | S25 | S33 | S33 |
| B | 2500 | 2500 | S2 | S2 | S10 | S10 | S18 | S18 | S26 | S26 | S34 | S34 |
| C | 1250 | 1250 | S3 | S3 | S11 | S11 | S19 | S19 | S27 | S27 | S35 | S35 |
| D | 630 | 630 | S4 | S4 | S12 | S12 | S20 | S20 | S28 | S28 | S36 | S36 |
| E | 310 | 310 | S5 | S5 | S13 | S13 | S21 | S21 | S29 | S29 | S37 | S37 |
| F | 160 | 160 | S6 | S6 | S14 | S14 | S22 | S22 | S30 | S30 | S38 | S38 |
| G | 80 | 80 | S7 | S7 | S15 | S15 | S23 | S23 | S31 | S31 | S39 | S39 |
| H | B | B | S8 | S8 | S16 | S16 | S24 | S24 | S32 | S32 | S40 | S40 |

Standards | Samples



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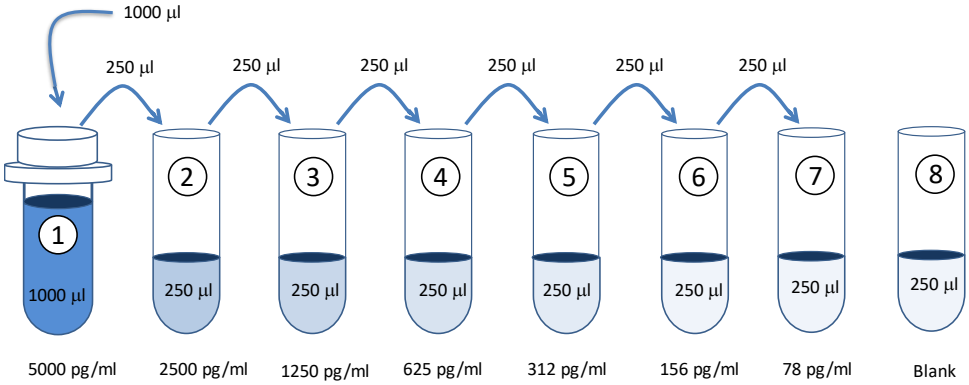
## ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: The recommended standard range is 78 - 5000 pg/ml. Dissolve one vial of Rat TNF-α standard in 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution B) for the 5000 pg/ml standard. Then serially dilute it with Solution B. For example, mix 250 µl of the standard (5000 pg/ml) with an equal volume of Solution B to make a 2500 pg/ml solution, and then repeat it five more times for 1250, 625, 312, 156, and 78 pg/ml solutions. The remaining 5000 pg/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.




2. **Prepare Samples:**

   **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 TNF-α level in the samples. Two to three different sample dilutions are recommended if the TNF-α levels in the samples are unknown.

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

3. **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.

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 Detection Antibody Solution**: Prepare the detection antibody solution with Sample/Standard/Detection Antibody Dilution Buffer (Solution B) as shown in the following table. 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 | 25 | 3.3 |
| 6 | 50 | 5.0 |
| 8 | 66 | 6.6 |
| 10 | 82 | 8.2 |
| 12 | 100 | 10.0 |

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 Streptavidin Peroxidase Solution**: Prepare streptavidin peroxidase solution with Streptavidin Peroxidase Dilution Buffer (Solution D) as shown in the following table. 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 | 17 | 1.7 |
| 4 | 25 | 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 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 |

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

11. **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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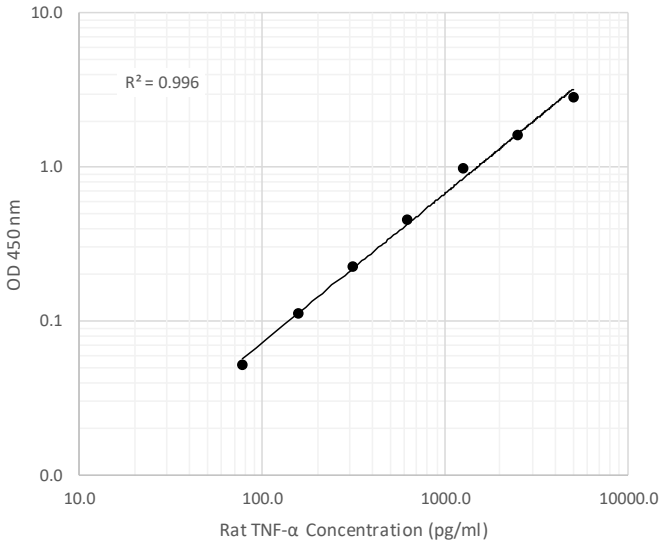
## CALCULATING RESULTS

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 Rat TNF-α (pg/ml). Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is 78 - 5000 pg/ml.
4. The pg/ml of TNF-α in test samples can be calculated using regression analysis.

Figure 1 - A Typical Standard Curve for the Rat TNF-α Detection ELISA Kit




## ASSAY VALIDATION

Table 1 - Reproducibility Data for the Rat TNF-α Detection ELISA Kit

| Test | 156 pg/ml | 625 pg/ml | 2500 pg/ml |
|---|---|---|---|
| Intra-Assay CV (%) | 21.0 | 3.1 | 1.1 |
| Inter-Assay CV (%) | 12.7 | 18.5 | 20.7 |
| Spike Test* (%) | 88% | 90% | 94% |

* Known amounts of Rat TNF-α were added to samples and then diluted with Sample/Standard/Detection Antibody Dilution Buffer (Solution B).

## TROUBLESHOOTING

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



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