TNFSF11 ELISA Kit Rat (OKAN04678)

Instructions for Use

For the quantitative measurement of TNFSF11 in Rat biological samples.

This product is intended for research use only.

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1. Background

Principle

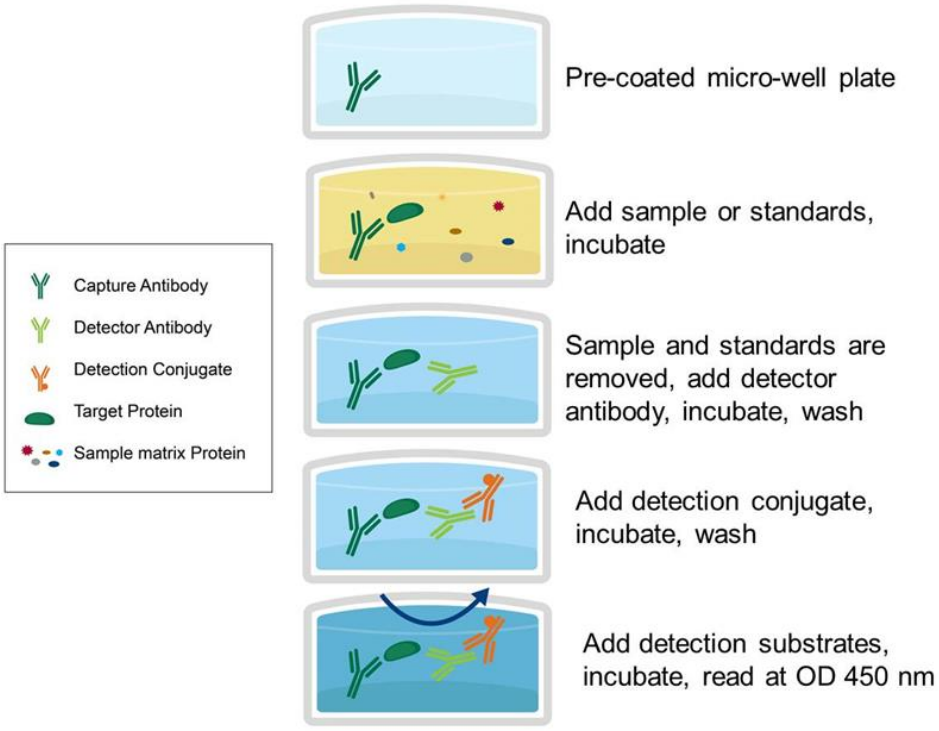
Aviva Systems Biology's TNFSF11 ELISA Kit (Rat) (OKAN04678) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for TNFSF11 has been pre-coated onto a 96-well plate (12 x 8 Well Strips). Standards or test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for TNFSF11 is added, incubated and followed by washing. Avidin-Peroxidase Conjugate is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is produced through the addition of TMB substrate which is catalyzed by HRP generating a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm is quantitatively proportional to the amount of sample TNFSF11 captured in the well.

Target Background

induces osteoclast formation and bone resoprtion; plays a role in regulation of calcium homeostasis and osteoclast function [RGD, Feb 2006]

General Specifications	
Specificity	Rat TNFSF11 UniProt: Q9ESE2 GeneID: 117516 Target Alias:
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

• Open kit immediately upon receipt. Store components at -20°C (NOTE: exceptions below) for long term storage of 6 months until expiration date. Avoid any freeze/thaw cycles.

4. Kit Components

• The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
TNFSF11 Microplate	96 Wells (12 x 8 strips)	-20°C for 6 months
TNFSF11 Lyophilized Standard	2 vials	Store at 4°C for 6 months
100X Biotinylated TNFSF11 Detector Ab	1 x 120 µL	
100X Streptavidin-HRP Conjugate	1 x 120 µL	
Sample Diluent (R1)	1 x 20 mL	
Biotin-Conjugate Antibody Diluent (R2)	1 x 12 mL	
Streptavidin-HRP Diluent (R3)	1 x 12 mL	
30X Wash Buffer	1 x 20 mL	
Stop Solution	1 x 6 mL	
TMB Substrate	1 x 9 mL	

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.
• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).
• Kit cannot be used beyond the expiration date on the label.

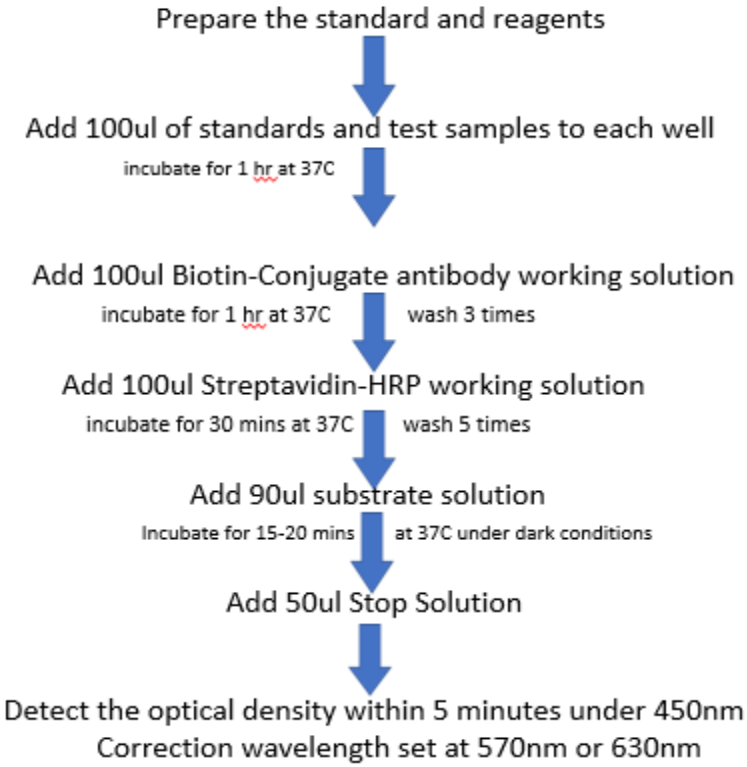
6. Required Materials Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.
• Automated plate washer (optional).
• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
• Absorbent paper or paper toweling.
• Distilled or deionized ultrapure water.
• 37°C Incubator (optional)

7. Technical Application Tips

• Do not mix or substitute components from other kits.
• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.
• Replicate wells are recommended for standards and samples.
• Cover microplate while incubating to prevent evaporation.
• Do not allow the microplate wells dry at any point during the assay procedure.
• Do not reuse tips or tube to prevent cross contamination.
• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
• Completely remove of all liquids when washing to prevent cross contamination.
• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
• Equilibrate all materials to ambient room temperature prior to use (standards exception).
• For optimal results for inter- and intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception) and perform all incubations at 37°C.
• Pipetting less than 1 µL is not recommended for optimal assay accuracy.
• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
• Samples containing bilirubin, precipitates or fibrin strands or are hemolytic or lipemic might cause inaccurate results due to interfering factors.
• TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

Assay Procedure Summary



8. Sample Preparation Guidelines

Sample Preparation and Storage

- Samples must be tested to determine if the kit is valid.
- Store samples to be assayed at 4°C for 24 hours prior being assayed.
- For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

General Sample Preparation Guidelines:

- **Serum** - Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1000x g, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles.
- **Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze / thaw cycles
- **Tissue Homogenates** – The preparation of tissue homogenates will vary depending upon tissue type. Tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed before homogenization. Minced the tissues to small pieces and homogenized them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular localization of the target protein) (e.g. 1mL lysis buffer is added in 200mg tissue sample) with a glass homogenizer on ice (Micro Tissue Grinders woks, too).The resulting suspension was sonicated with an ultrasonic cell disrupter till the solution is clarified. Then, the homogenates were centrifuged for 5 minutes at 10,000×g. Collect the supernates and assay immediately or aliquot and store at ≤-20°C

- **Cell Lysates -** Cells need to be lysed before assaying according to the following directions. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000 × g for 5 minutes (suspension cells can be collected by centrifugation directly). Wash cells three times in cold PBS. Resuspend cells in fresh lysis buffer with concentration of 107 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified. Centrifuge at 1,500×g for 10 5 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤-20°C.
- **Cell culture supernatants and other biological fluids -** Centrifuge 1000x g for 10 min and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles. If cell culture supernate samples require larger dilutions, perform an intermediate dilution with culture media and the final dilution with the Standard/Sample Diluent

Recombinant Proteins: Due to the possibility of mismatching between antigen from other origin and antibody used in our kits (e.g. antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products

9. Reagent Preparation

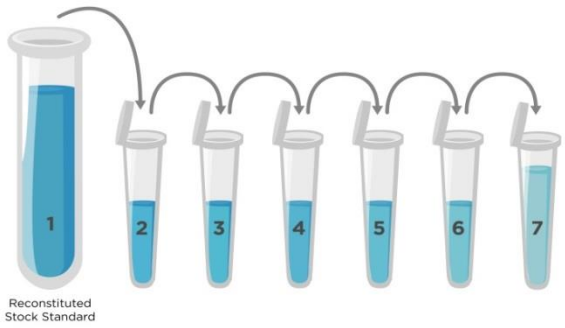
- Equilibrate all materials to room temperature prior to use and use immediately.

9.1. Standard

9.1.1. Prepare a fresh standard curve for each assay performed. Reconstituted standards cannot be stored for later use. For further directions, please refer to the Certificate of Analysis.

9.1.2. Add **Sample Diluent**(R1) 1.0mL into freeze-dried standard, sit for a minimum of 15 minutes with gentle agitation prior to making dilutions (20 ng/mL),

9.1.3. Prepare tubes containing **Sample Diluent**(R1), and produce a dilution series according to the picture shown below (recommended concentration for standard curve: 100ng/mL, 50ng/mL, 25ng/mL, 12.5 ng/mL, 6.25ng/mL, 3.12ng/mL, 1.56ng/mL). Redissolve standard solution (200ng/mL), aliquot and store at -20°C — - 70°C.



9.2. 1X Biotinylated TNFSF11 Detector Antibody

9.2.1. Prepare the **1X Biotinylated TNFSF11 Detector Antibody** immediately prior to use by diluting the **100X Biotinylated TNFSF11 Detector Antibody** 1:100 with **Detector Antibody Diluent**.

9.2.2. For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Biotinylated TNFSF11 Detector Antibody** to 990 µL **Detector Antibody Diluent**.

9.2.3. Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

Strip	Concentrated Biotin-Conjugate antibody (100x)	Biotin-Conjugate Antibody Diluent (R2)
2	20ul	1980ul
4	40ul	3960ul
6	60ul	5940ul
8	80ul	7920ul
10	100ul	9900ul
12	120ul	11880ul

9.3. 1X Avidin-HRP Conjugate

9.3.1. Prepare the **1X Avidin-HRP Conjugate** immediately prior to use by diluting the **100X Avidin-HRP Conjugate** 1:100 with **Conjugate Diluent**.

9.3.2. For each well strip to be used in the experiment (8-wells) prepare 1,000 µL by adding 10 µL of **100X Avidin-HRP Conjugate** to 990 µL **Conjugate Diluent**.

9.3.3. Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

9.4. Streptavidin-HRP Concentrated (100x):

9.4.1. Dilute 1:100 with the Streptavidin HRP Diluent(R3) before use, and the diluted solution should be used within 30 min.

Strip	Concentrated Streptavidin-HRP (1:100)	Testing dilution buffer (R3)
2	20	1980
4	40	3960
6	60	5940
8	80	7920
10	100	9900
12	120	11880

9.5. 1X Wash Buffer

If crystals have formed in the **25X Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

9.5.1. Add the entire 30 mL contents of the **25X Wash Buffer** bottle to 720 mL of ultra-pure water to a clean > 1,000 mL bottle or other vessel.

9.5.2. Seal and mix gently by inversion. Avoid foaming or bubbles.

9.5.3. Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

9.6. Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9.7. Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted.

• Prior to performing the full experiment, test a serially diluted representative sample from your cohort.

- Alternately, a small pool of several samples can also be used with this same method if sample volume is limited.

-or-

- Refer to published literature for expected concentrations and derive the optimal dilution level based on the expected dynamic range of the kit

• Dilute samples using **Sample Diluent**.

• Mix diluted samples gently and thoroughly.

• Pipetting less than 2 µL is not recommended for optimal assay accuracy.

10. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Optimal results for intra- and inter-assay reproducibility will be obtained when performing incubation steps at 37°C as indicated below.

10.1. Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

10.2. Add 100 µL of serially titrated standards, diluted samples or blank into wells of the **TNFSF11 Microplate**. At least two replicates of each standard, sample or blank is recommended.

10.3. Cover the plate with the well plate sealer and incubate at 37°C for 2 hours.

10.4. Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.5. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.6. Add 100 µL of prepared **1X Biotinylated TNFSF11 Detector Antibody** to each well.

10.7. Cover with the well-plate sealer and incubate at 37°C for 60 minutes.

10.8. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.9. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.10. Wash plate 3 times with **1X Wash Buffer** as follows:

10.10.1. Add 300 µL of **1X Wash Buffer** to each assay well.

10.10.2. Incubate for 1 minute.

10.10.3. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.10.4. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.10.5. Repeat steps 10.10.1 through 10.10.4 **two** more times.

10.11. Add 100 µL of prepared **1X Avidin-HRP Conjugate** into each well, cover with plate sealer and incubate at 37°C for 60 minutes.

10.12. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.13. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.14. Wash plate **5 times** with **1X Wash Buffer** as in Step 10.10.

10.15. Add 90 µL of **TMB Substrate** to each well, cover with plate sealer and incubate at 37°C in the dark for 10-20 minutes. Wells should change to gradations of blue. If the color is too deep, reduce the incubation time.
(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)

10.16. Add 50 µL of **Stop Solution** to each well. Well color should change to yellow immediately. Add the **Stop Solution** in the same well order as done for the **TMB Substrate**.

10.17. Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 10.16. If wavelength correction is available, set to 570 nm or 630nm.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative OD_{450}** for each test or standard well as follows:
(Relative OD_{450}) = (Well OD_{450}) – (Mean Blank Well OD_{450})

The standard curve is generated by plotting the mean replicate **Relative OD_{450}** of each standard serial dilution point vs. the respective standard concentration. The concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD_{450}** against the standard curve. This is best achieved using curve fitting software.

Note: if wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Precision

Intra-plate Precision
3 samples with low, middle and high levels TNFSF11 were tested on 3 different plates, 8 replicates on each plate
Inter-assay: CV<10%

Inter-plate Precision
3 samples with low, middle and high TNFSF11 were tested on 3 different plates, 8 replicates in each plate.
Inter-assay: CV<12%

13. Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

USA

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