

## MFGE8 (RAT/MOUSE) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF RAT AND MOUSE MFGE8 CONCENTRATIONS IN MILK, PLASMA AND SERUM



ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

### PRODUCT INFORMATION:

ELISA NAME	RAT/MOUSE MFGE8 ELISA
Catalog No.	SK00649-02
Formulation	96 T
Standard range	312.5 ~20000 pg/mL
Sensitivity	50 pg/mL
Sample Volume	100 µL
Dilution Factor	<b>Optimal dilutions should be determined by each laboratory for each application</b>
Sample Type	Milk, Serum, Plasma
Specificity	Rat, Mouse
Calibration	Rat MFGE8 recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8 °C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

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## DESCRIPTION

This Rat/Mouse Milk fat globule-EGF factor 8 (MFGE8) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural rat MFGE8 from milk, serum and plasma in a sandwich ELISA format. This immunoassay contains recombinant rat MFGE8 and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural MFGE8 in rat and mouse samples.

## ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for rat MFGE8. The capture antibody can bind to the rat MFGE8 in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against rat MFGE8 is added to the wells. After another washing of the plate, the streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of rat MFGE8 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

## PROCEDURAL LIMITATIONS

\_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

\_This ELISA kit should not be used beyond the expiration date on the kit label.

\_Do not mix reagents with those from other lots or sources.

\_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

\_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.

\_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.

\_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

## COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
<b>Rat MFGE8 Microplate</b> – 96 well microplate coated with antibody specific for rat MFGE8.	<b>649-02-01</b>	<b>1 plate</b>
<b>Rat MFGE8 Standard</b> – 100 ng/vial of lyophilized recombinant <b>rat MFGE8</b>	<b>649-02-02</b>	<b>1 vial</b>
<b>Detection Antibody Concentrate</b> – 1.2 mL/vial of 10-fold concentrate of lyophilized biotinylated antibody against <b>rat MFGE8</b> .	<b>649-02-03</b>	<b>1 vial</b>
<b>Positive Control</b> – one vial of lyophilized recombinant <b>rat MFGE8</b>	<b>649-02-04</b>	<b>1 vial</b>
<b>Streptavidin-HRP Conjugate</b> – 120 µL/vial of 100-fold concentrated solution of Streptavidin-HRP conjugate.	<b>SAHRP</b>	<b>1 vial</b>
<b>Dilution Buffer</b> – 60 mL of buffered solution with preservative.	<b>DB01</b>	<b>1 bottle</b>
<b>HRP Diluent Solution</b> – 12 mL of buffered solution with preservative.	<b>DB08</b>	<b>1 bottle</b>
<b>Wash Buffer</b> – 50 mL of 10-fold concentrated buffered surfactant with preservative.	<b>WB01</b>	<b>1 bottle</b>
<b>TMB Substrate Solution</b> – 11 mL of TMB substrate solution.	<b>TMB01</b>	<b>1 bottle</b>
<b>Stop Solution</b> – 11 mL of 0.5M HCl.	<b>S-STOP</b>	<b>1 bottle</b>
<b>Plate Sealer</b>	<b>EAPS</b>	<b>1 piece</b>
<b>Plastic Pouch</b>	<b>P01</b>	<b>1 piece</b>

## STORAGE

**Unopened Kit:** Store at 2 – 8 °C for up to 8 months. For longer storage, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 °C or -70 °C. Do not use kit past expiration date.

**Opened / Reconstituted Reagents:** Reconstituted Standard (stock) solution and Detection Antibody concentrated solution **SHOULD BE STORED** at -20 °C or -70 °C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrated solution and TMB

Substrate Solution can be stored at 2 – 8 °C for up to 8 months (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). All other components may be stored at 2 – 8 °C for up to 8 months.

**Microplate Wells:** Return unused strips to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8 °C after opening.

**ADDITIONAL MATERIALS REQUIRED**

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

**PRECAUTION**

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

**SAMPLE COLLECTION AND STORAGE**

**Serum** – Use a serum separator tube (SST). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per mL of sample solution.**

**SAMPLE PREPARATION**

Serum and Plasma samples may require dilutions.

**Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.**

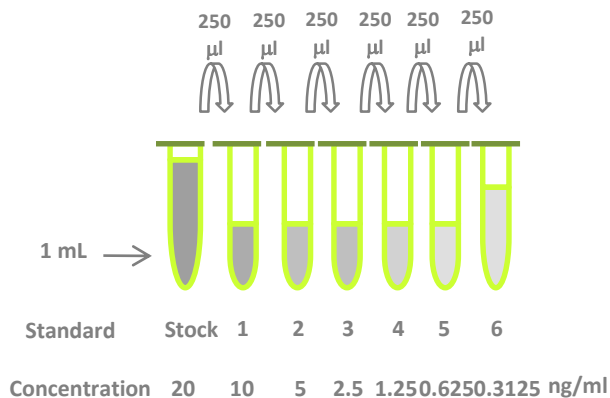
**REAGENT PREPARATION**

**Bring all reagents to room temperature before use.**

**Wash Buffer** – Dilute 50 mL of Wash Buffer Concentrate into 450 mL distilled or deionized water to make 500 mL of 1x Wash Buffer. If crystals have formed in the concentrate, warm bottle in a water bath until the crystals have completely dissolved.

**Reat MFGE8 Standard** – Reconstitute the human Pro BDNF standard with 1.0 mL of Dilution Buffer. The concentration of the reconstituted stock solution is 20 ng/mL. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	1.0 mL	20 ng/mL
# 1	250µL of stock	250µL	10 ng/mL
# 2	250µL of 1	250µL	5 ng/mL
# 3	250µL of 2	250µL	2.5 ng/mL
# 4	250µL of 3	250µL	1.25 ng/mL
# 5	250µL of 4	250µL	0.625 ng/mL
# 6	250µL of 5	250µL	0.3125 ng/mL



**Detection Antibody** - Reconstitute the Detection Antibody Concentrate with 1.2 mL of Dilution Buffer to produce a 10-fold concentrated stock solution. Allow the concentrated solution to sit for at least 5 minutes until completely dissolved. Pipette 10.8 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 10.8 mL of 10-fold concentrated stock solution to prepare working solution.

**Streptavidin-HRP Conjugate** - Pipette 11.88 mL of HRP Diluent Solution into a 15 mL centrifuge tube and transfer 120  $\mu$ L of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of streptavidin-HRP should be used within a few days (**protect from light**). **DO NOT FREEZE.**

**Positive Control** - Reconstitute the Positive Control with 1.0 mL Dilution Buffer. **Note:** Positive Control could be used within a few days if stored at -20 °C or -70 °C.

### ELISA PROTOCOL

**Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.**

1. Prepare all reagents, standard dilutions, positive control and samples as directed previously.
2. Remove unneeded microplate strips from the plate frame and return them to the plastic pouch with the desiccant pack.
3. Add 100  $\mu$ L per well of **Dilution Buffer** to Blank wells (A2, A3).
4. Add 100  $\mu$ L per well of **Standard Dilutions** in reverse order of serial dilution (B2, B3 to H2, H3), **sample**, or **positive control** (C4, C5). Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature. (Please see plate layout provided.)
5. Aspirate and wash each well with 300  $\mu$ L of **1x Wash Buffer** four times. After the last wash, aspirate any remaining 1x Wash Buffer, invert the plate and blot against clean paper towel(s).
6. Add 100  $\mu$ L per well of **Detection Antibody working solution**. Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration and wash as in step 5.
8. Add 100  $\mu$ L per well of **Anti Rabbit IgG-HRP Conjugate working solution**. Cover with plate sealer and incubate for 40 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration and wash as in step 5.
10. Add 100  $\mu$ L per well of **Substrate Solution**. Incubate for 15 minutes on microplate shaker at room temperature. **Protect from light.**

11. Add 100  $\mu$ L per well of **Stop Solution**. The color in the wells should change from blue to yellow. If the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Read plate using a microplate reader set to 450 nm within 15 minutes.

### CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

### SPECIFICITY

PROTEIN	CROSS-REACTIVITY
Rat MFGE8	100%
Human MFGE8	0
Rat Adiponectin	0
Mouse Betatrophin	0
Mouse FGF-21	0
Mouse CTRP15	0

The data also indicated that mouse serum and EDTA plasma samples were competitively bound to antibody that was used in this kit formulation condition. Its linear dilution curves were parallel to the standard curves obtained using the ELISA standard. That means mouse samples cross-react with rat MFGE8 ELISA kit.

**TYPICAL STANDARD CURVE**

This standard curve is for demonstration only. A new standard curve should be made for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)
Blank	0 (0.103)
312.5	0.052
625	0.108
1250	0.207
2500	0.401
5000	0.702
10000	1.346
20000	2.178

**SUMMARY OF ASSAY PROCEDURE**

