MOUSE SOLUBLE CSF1R/CD115 ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF MOUSE CSF1R CONCENTRATIONS IN EDTA PLASMA AND SERUM

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

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

PRODUCT INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	MOUSE SOLUBLE CSF1R/CD115 ELISA KIT
Catalog No.	SK00144-03
Lot No.	20114856
Formulation	3 x 96 T
Standard range	31.25 ~ 2000 pg/mL
Sensitivity	10 pg/mL
Sample Volume	10 µL
Dilution Factor	1600 ~ 3200 (Optimal dilutions should be determined by each laboratory)
Sample Type	Serum, EDTA Plasma
Specificity	Mouse Soluble CSF1R
Calibration	Mouse Soluble CSF1R Recombinant (HEK293 derived)
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 9%
Storage	2 – 8° C for 6 months, more information check page 2-3
This kit contains sufficient materials to run approximately 35~40 samples duplicated provided that assay is run according to protocol.	

Order Contact:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Email: Sales@AvisceraBioscience.com
www.AvisceraBioscience.com
www.AvisceraBioscience.net

DESCRIPTION

This Mouse Soluble CSF1R ELISA Kit contains the necessary components required for the quantitative measurement of recombinant mouse sCSF1R (HEK293 derived) and/or natural mouse CSF1R from plasma, serum samples in a sandwich ELISA format.

This immunoassay contains mouse sCSF1R recombinant and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify bioactive recombinant and natural mouse CSF1R in the samples. Rat EDTA plasma and serum was detectable by this ELISA Kit.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for mouse CSF1R. The Assay Solution DB91Y (Yellow Color) and Antibody Diluent Solution DB0591G (Green Color) as universal blocking reagents was used in the assay wells to reduce matrix interference if the samples containing heterophilic antibodies (HAMA and rheumatoid factor). The capture antibody can bind to the mouse CSF1R in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against mouse CSF1R is added to the wells. After another washing of the plate, 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 mouse CSF1R 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.
_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
CSF1R Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a antibody against mouse CSF1R.	**144-03-01**	**1 plate**
CSF1R Standard – 32 ng/vial of recombinant mouse CSF1R in a buffered protein base with preservative; lyophilized.	**144-03-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial of 10-fold concentrate of biotinylated antibody against mouse CSF1R with preservative; lyophilized.	**144-03-03**	**1 vial**
Positive Control Concentrated - one vial of recombinant mouse CSF1R; lyophilized.	**144-03-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Assay Solution - 12 mL of **Yellow Color** universal blocking solution.	**DB91Y**	**1 bottle**
Dilution Buffer - 45 mL of **Blue Color** buffered protein based solution with preservative.	**DB05B**	**2 bottles**
Antibody Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB12**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservative.	**DB08B**	**1 bottle**
Wash Buffer 20X - 25 mL of 20-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**

Stop Solution - 11 mL of 0.25M HCl solution.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

SK00144-03 (3 plates of 96T) was packaged with 3 boxes of 96T.

STORAGE

Unopened Kit: Store at 2 – 8° C up to 6 months. For longer storage up to 10 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20° C.
The Assay Solution, Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2 ~ 8°C.
Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (200 – 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) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma – Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge at 1000 x g for 15 minutes and collect plasma. 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

Mouse EDTA Plasma or serum samples require 1600 ~ 3200 fold dilution.
A suggested 160-fold dilution is 5 µl sample + 795 µl Dilution Buffer DB05B.
A suggested 1600-fold dilution is 10 µl of 160-fold diluted sample per well + 90 µl of Dilution Buffer DB05B per well.
A suggested 3200-fold dilution is 5 µl per well of 160-fold diluted sample + 95 µl per well of Dilution Buffer DB05B.

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

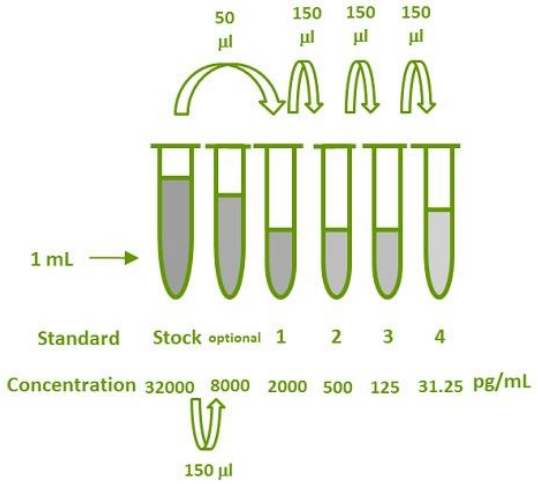
REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer 20X - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 25 mL of Wash Buffer Concentrate 20X into deionized or distilled water (475 mL) to prepare 500 mL of 1x Wash Buffer.

CSF1R Standard - Reconstitute the CSF1R standard with 1.0 mL of Dilution Buffer DB05B. This reconstitution produces a stock solution of 32ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Use the stock solution to produce a 4-fold dilution series (below). Mix each tube thoroughly before the next transfer. The **2000 pg/mL** standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL). The optional set 8000 pg/ml as the highest standard concentration to fit standard curve by 4-parameter.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0ml	32 ng/ml
optional	150 µl of stock	450 µl	8000 pg/ml
# 1	50 µl of stock	750 µl	2000 pg/ml
# 2	150 µl of 2	450 µl	500 pg/ml
# 3	150 µl of 3	450 µl	125 pg/ml
# 4	150 µl of 4	450 µl	31.25 pg/ml



Positive Control - Reconstitute the Positive Control with 1 mL of Dilution Buffer to prepare the working solution of positive control. Discard the positive control and its tock solution after use. It is for one time use only.

Detection Antibody - Reconstitute the Detection Antibody Concentrate with 1.2 mL of **Antibody Diluent Solution (DB12)** to produce a 10-fold concentrated stock solution. For 96 wells test, freshly pipette 9.45 mL of **Antibody Diluent Solution (DB12)** into a 15 ml centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution. *If run a partial strip test, freshly prepare 900 µL per strip (8-wells) of working solution. Store the stock solution of 10-fold concentrated detection antibody at -20 °C for a few days.*

Streptavidin-HRP Conjugate - Pipette 10.89 mL of **HRP Diluent Solution (DB08B)** into a 15 mL centrifuge tube and transfer 110 µL of 100-fold concentrated stock solution to prepare working solution **(protect from light).** *The working solution of Streptavidin-HRP Conjugate should be freshly prepared and used within 10-20 min. If run a partial strip test, freshly prepare 900 µL per strip (8-wells) of working solution. Store the stock solution of 100-fold concentrated Streptavidin HRP ONLY at 2 -8°C for 12 months.*

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 and working standards as directed in the previous sections.
2. Add 100 µL per well of the **Yellow Color Assay Solution DB91Y** to each assay well (total 96 wells) by Multi-channel Pipette. Cover the plate with seal. **Incubate it for 10 minutes on microplate shaker (250 rpm) at room temperature prior to next step**.
3. Add 100 µL per well of **Blue Color Dilution Buffer DB05** to Blank wells. Add 100 µL per well of **Blue Color standard dilutions to standard well.** Add 100 µL per well of Blue Color Positive Control to PC well. Add 100 µL per well of **Blue Color diluted samples** to each sample well. All mixed assay solutions in each assay well appear to light green color. Cover with the plate sealer. Incubate for **2 hours** on microplate shaker at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with 1x Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL per well of Detection Antibody working solution to each well. Cover with the plate sealer. Incubate for 2 hours on microplate shaker (250 rpm) at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL per well of Streptavidin-HRP Conjugate working solution to each well. Incubate for 45 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL per well of Substrate Solution to each well. Incubate for 15~ 20 minutes on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL per well of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well using a microplate reader set to 450 nm within 3 min.

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 or 4-Parameter 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.

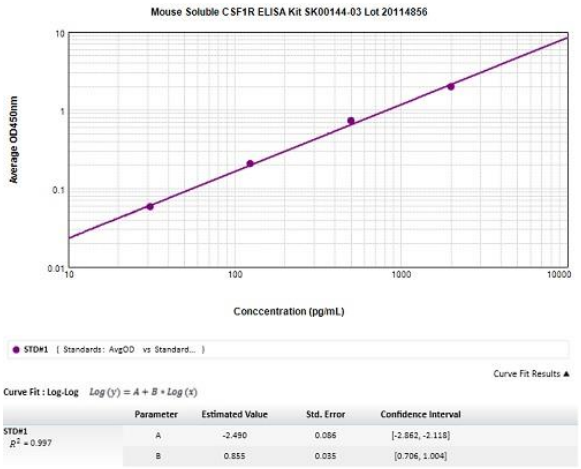
TYPICAL STANDARD CURVE

This standard curve is provided for demonstration only. A new standard curve with specific lot should be generated for each set of samples assayed.

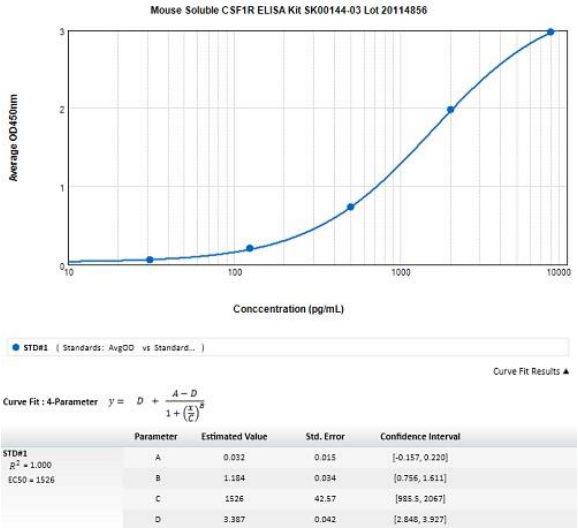
STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.084)
31.25	0.058
125	0.207
500	0.753
2000	1.928
8000 (optional)	2.819

Lot No.: 20114856
Positive Control: 500 ~ 2000 pg/mL

Standard curve (31.25 ~ 2000 pg/mL) by log-log fit:



Standard curve with (31.25 ~ 8000 pg/mL by 4-parameter fit:

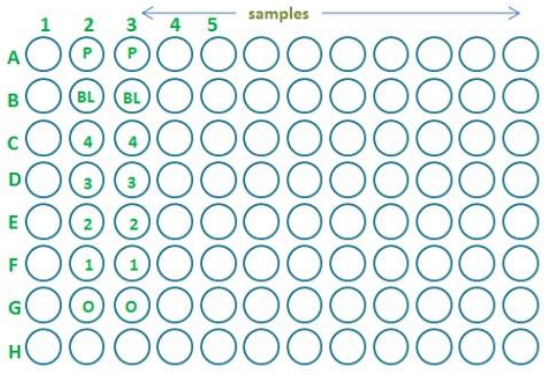


SPECIFICITY

PROTEINS	CROSS-REACTIVITY
Mouse CSF1R (HEK293 derived)	100%
Human CSF1R (HEK293 derived)	0

The 32ng/ml of human soluble CSF1R (HEK293 derived) in Dilution Buffer DB05B was assayed at 21.7 pg/mL which is out of assay range.

The rat EDTA plasma and serum samples pre-diluted by 10 ~ 40 fold in Dilution Buffer DB05B were detectable by this ELISA Kit. However, the cross-reactivity of rat CSF1R recombinant (HEK293) should be determined by each laboratory for each application.



SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
⬇
Add 100 µl per well of the **yellow color** of Assay Solution to each assay well. Add 100 µl per well of the **blue color** standard dilutions, diluted samples, or positive control to each well. **All mixed assay solutions in each well appear to Green Color**. Incubate **2 hours** on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl per well of **Detection Antibody working solution** to each well. Incubate 2 hours on the plate shaker at RT.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Streptavidin HRP conjugate working solution to each well. Incubate 45 min on the plate shaker at RT. **Protect from light**.
⬇
Aspirate and wash 4 times.
⬇
Add 100 µl Substrate Solution to each well. Incubate 10-15 min on the plate shaker at RT. **Protect from light**.
⬇
Add 100 µl Stop Solution to each well. Read at 450 nm within 3 min.

Mouse Samples Test

The research pooled mouse samples were diluted by Dilution Buffer DB05B and assayed by Mouse sCSF1R ELISA Kit Sk00144-03

Sample	Dilution Factor	Assayed (ng/mL)	Final (µg/mL)	Recovery (%)
EDTA Plasma	1600	1.673	2.676	100
EDTA Plasma	6400	0.434	2.774	104
Serum	1600	5.787	9.259	100
Serum	6400	1.529	9.785	106