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Monkey GM-CSF ELISA Matched Antibody Set

Catalog No: CK1020 **Lot:** TBD **Size:** 10-plate format **Expiration Date:** 1 year from receipt

Specifications:

Range:	2-128 pg/ml
Sensitivity:	2 pg/ml
Specificity:	Validated for Native Cynomolgus Monkey, Rhesus Macaque GM-CSF
Type of sample:	Plasma, serum, and cell culture supernatant

Introduction:

The usefulness of ELISAs (enzyme-linked immunosorbent assays) in cytokine biology is evident from the many reports published on this subject. The assay requires two antibodies (either mono or polyclonal antibodies) that bind with high affinity and specificity to different epitopes of the analyte of interest. One of the antibodies is immobilized to the wells of a 96-well microtiter plate. This coating antibody, functions to selectively immobilize the analyte from crude protein preparations. The second antibody (detection antibody) is labeled with biotin and binds to another epitope of the analyte. Biotin allows the antibody to interact with streptavidin molecules. By using HRP (horseradish peroxidase)-labeled streptavidin, the analyte can be quantified using the enzymatic conversions of an HRP-specific substrate (i.e. TMB). The results are a sensitive assay with a detection limit within the low picogram range.

Reagents Provided:

Reagent	Quantity	Storage
CK1020-A Coating Antibody, Mouse mAb IgG1 anti-monkey GM-CSF	2 vials	2-8 °C
CK1020-B Biotinylated Detection Ab, Rat mAb IgG2a anti-monkey GM-CSF	2 vials	2-8 °C
CK1020-C Standard, Recombinant rhesus macaque GM-CSF **13.5 ng/vial**	5 vials	2-8 °C
CK1020-D SPP Conjugate (Streptavidin-HRP)	2 vials	-20 °C in the dark

Reagents and Equipment Required:

- Bovine serum albumin (BSA; ELISA grade)
- Phosphate Buffered Saline (PBS), pH 7.4
- Pipetting device(s) for the accurate delivery of volume required for the assay performance
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
- Reading device for microtiter plates with detection range suitable for selected substrate
- Sterile distilled water
- Tubes, containers, to prepare solutions, adhesive cover slips
- Stop Solution H_2SO_4 [recommend 0.18 M]
- TMB Substrate Solution
- Tween-20 detergent

Cell Sciences
65 Parker Street
Unit 11
Newburyport, MA 01950
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Phone: 978-572-107
Fax: 978-992-0298
E-mail: info@cellsciences.com
Website: www.cellsciences.com

- 96-well ELISA plates with adhesive cover strips
- 37°C incubator

Note: Do not use commercially available PBS tablets for the preparation of the coating solution (the filler in the tablets interferes with the coating process).

Reagent Preparation:

- **PBS**: 5.4 mM Na_2HPO_4 + 1.3 mM KH_2PO_4 + 150 mM NaCl, pH7.4
- **Wash Buffer**: 500µL (0.5 mL) Tween-20 + 1 L PBS
- **Blocking Buffer**: PBS + 1% (w/v) BSA
- **Dilution Buffer**: PBS + 0.5% (w/v) BSA + 0.05% (w/v) Tween-20
- **Coating Antibody**: Add **250 µL as indicated on the vial of** sterile distilled water to the vial. Mix gently for 15 seconds and allow to stand for 5 minutes at RT. Do not vortex. Dilute 100-fold in PBS enough for 50µl/well
- **Detection Antibody**: Add **500 µL as indicated on the vial of** sterile distilled water to the vial. Mix gently for 15 seconds and allow to stand for 5 minutes at RT. Do not vortex. Dilute 100-fold in **Dilution Buffer** enough for 100µl/well.
- **SPP Conjugate (Streptavidin-HRP):** Add **500 µL** sterile distilled water to the vial. Mix gently for 15 seconds and allow to stand for 5 minutes at RT. Do not vortex. Dilute 100-fold in PBS + 0.5% BSA + 0.05% Tween 20 = 100µl/well (5 x 96 well plates).
- **Standard**: add **500 µL** sterile distilled water to vial and mix gently for 15 seconds, then allow to stand for 5 minutes at RT. Do not vortex. Place on ice. Dilute immediately (within 1 hour) in Dilution Buffer to desired concentrations to be used in the standard curve range. Discard vial after use (1 hour).
- In general, when TMB substrate is used, the linear portion of the curve falls within the range of 2-100pg/mL.

Assay Procedure:

Preparation of Samples:

- Samples should be clear, non-hemolyzed and non-lipemic. Excessive hemolysis and the presence of large clots or microbial growth in the sample may interfere with the performance of the test.
- Dilute Samples in Dilution Buffer, at least 2-fold (1:1)
- Avoid repeated freeze/thaw cycles of Samples.
- The diluent for the Standard and Blank control should preferentially be control serum or plasma originating from the same species. For measuring cytokines in cell culture supernatant, samples should be diluted in Dilution Buffer.

Preparation of Standards:

With a standard curve, the analyte concentrations in the unknown samples can be determined. The standard curve is generated from the data of **7 two-fold serial dilutions (Std 1-7)** of the Standard. The recommended assay range for the Monkey GM-CSF ELISA set is **2-128 pg/ml**. It is recommended to test the standard dilutions (Std 1-7) in triplicate (see figure on next page). **Actual assay performance will vary due to individual user and laboratory conditions.**

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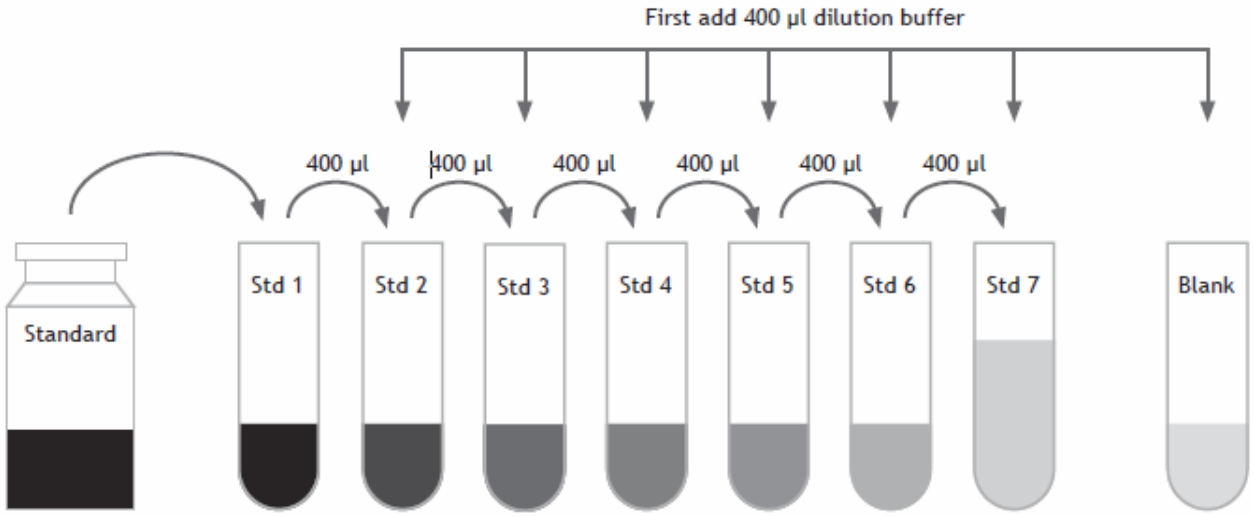

- Take 8 tubes and add 400 µl Dilution buffer to 7 of these tubes (Std 2 to Std 7 and Blank).
- Prepare in the remaining tube (Std 1) the highest concentration to be used in the standard curve range (see **Specifications Table**) by mixing an appropriate volume of Standard with Dilution buffer. The final volume of Std 1 should be 800 µl. Allow the mixture to stand for at least 15 seconds before using in further dilutions.
- Perform two-fold serial dilutions: transfer 400 µl diluted standard from Std 1 to the next tube (Std 2), mix well and repeat this step until Std 7.

Directions for washing

- Incomplete washing of the wells will adversely affect the assay. All washing steps should be performed with Wash buffer.
- Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering the tip of an aspiration device into each well (without touching the bottom). After aspiration, fill the wells with at least 250 µl Wash buffer and then aspirate the liquid. Repeat these steps at least six times. After washing, the plate is inverted and tapped dry on absorbent tissue paper. Alternatively, the Wash buffer may be put into a squirt bottle. If a squirt bottle is used, empty the wells and flood the plate with Wash buffer, completely filling all wells. Repeat these steps at least six times. After washing, the plate is inverted and tapped dry on absorbent tissue paper.
- When using an automated washing device, follow operating instructions carefully.

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Method: ***Note: All solutions should be at RT prior to use.***

1. Pipette 50 µL of diluted Coating Antibody into the wells of a 96-well ELISA plate. Fill up to 100 µL with PBS.
2. Seal the plate with an adhesive cover slip and incubate overnight at 2-8° C.
3. Aspirate Coating Antibody solution and wash the wells at least 6 times with Wash Buffer.
4. Add 200 µL Blocking Buffer to each well.
5. Seal the plate with an adhesive cover slip and incubate 1 hour at 37° C.
6. Dilute Samples and Standards in Dilution Buffer.
7. Remove Blocking Buffer by a vigorous Shake-Out action but do not wash plate.
8. Add 100 µL of diluted Samples and Standards to the appropriate well(s).
9. Seal the plate and incubate for 2 hours at 37° C or overnight at 2-8° C.
10. Aspirate Samples and Standards and wash the wells at least 6 times with Wash Buffer.
11. Add 100 µL of diluted Detection Antibody to each well.
12. Seal the plate with an adhesive cover slip and incubate 1 hour at 37° C.
13. Aspirate Detection Antibody and wash the wells at least 6 times with Wash Buffer.
14. Add 100 µL of diluted SPP Conjugate to each well.
15. Seal the plate with an adhesive cover slip and incubate 1 hour at 37° C.
16. Aspirate the Conjugate and wash the wells at least 6 times with Wash Buffer.
17. Add an HRP- specific substrate solution to each well. The volume and incubation conditions depend on the type and brand of substrate used. Typically, with a TMB substrate solution, the plate is incubated at room temperature in the dark for about 15 to 25 minutes. The chromogenic solution produces a blue-colored product, which can be read at 370 nm or 655 nm.
18. Stop the reaction when possible. When TMB substrate solution is used, add 100 µL of 0.18 M H_2SO_4, resulting in a yellow color that can be read at 450 nm within 30 minutes.

Interpretation of Results

In each experiment, a standard curve needs to be run, consisting of 7 standard dilutions, from which a concentration-response relationship is generated. Construct the standard curve by plotting the mean OD of each standard dilution (Std 1-7) minus the mean Blank (see formula below) on the y-axis against the corresponding concentration on the x-axis.

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Formulas: Calculated adjusted Standard OD = mean OD of Standard – mean OD of the Blank for each Standard.

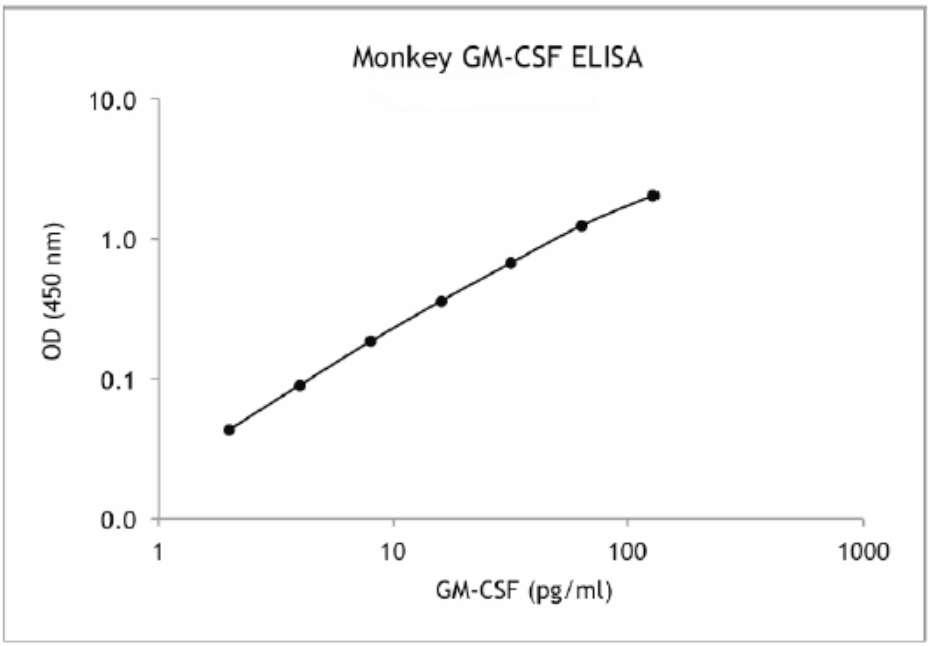
Calculated adjusted Sample OD = mean OD of Sample – mean OD of the Blank for each Sample.

Most laboratories have (plate reader) software that allows various methods of curve fitting. Since ELISA data are essentially sigmoidal rather than linear, we recommend using the 4- or 5-parameter logistic fit for quantitative analysis of the samples. Alternatively, a linear regression curve may be acceptable for the linear portion of the curve consisting of at least 3 concentrations.

After selection of the regression model, the analyte concentrations in unknown samples can be interpolated from the standard curve. The OD value of the sample should fall within the standard curve. Samples showing an OD below the lowest concentration of the standard curve need to be re-analyzed at a lower dilution. Samples showing an OD that exceeds the highest concentration of the standard curve need to be re-analyzed at a higher dilution.

If samples have been diluted, the calculated concentration must be multiplied by the dilution factor.

Typical Data - Actual assay performance will vary due to individual user and laboratory conditions.



Example of a representative Monkey GM-CSF standard curve using a 4-parameter logistic fit.

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Troubleshooting

Problem	Possible cause	Solution
Poor consistency of replicates	Inaccurate pipetting	- Ensure accurate pipetting of volume and avoid air bubbles. - Check pipettes.
	Inadequate mixing of reagents	- Mix reagents adequately.
	Inadequate washing	- Increase the stringency of washes (particularly after the 'detection antibody' incubation step). - Check function of the plate washer.
	Evaporation of solutions	- Ensure precise sealing of the plate.
	Non-homogenous samples or with high particulate matter	- Mix samples thoroughly and remove particulates by centrifugation.
OD_{blank} values higher than 0.3	Incubation time of TMB substrate solution is too long	- Shorter incubation time of TMB substrate.
	Incubation temperature of TMB substrate solution is too high	- Decrease incubation temperature of TMB substrate.
	Working solutions were contaminated	- Solutions should be clear and colorless. Use a clean container before addition into wells.
	Detection antibody or conjugate dilution was too concentrated or left too long on the plate	- Ensure proper dilution of detection antibody or conjugate and incubation time.
No signal or low OD values for standards	Improper storage of reconstituted SPP	- Avoid prolonged exposure to light and heat. - Avoid storage at RT.
	Incorrect incubation times or temperature	- Ensure sufficient incubation times. - Reagent solutions should be at RT before use.
	Improper quality or pH of distilled water	- Use distilled water, and not tap water. - Check quality and pH of distilled water.
	Improper antibody, SPP or standard dilution	- Ensure proper dilution of antibody, SPP and standard.
	Degradation of antibodies or SPP	- Follow recommended storage conditions.
	Overly high washing / aspiration pressure from automated plate washer.	- Check function of washing system or utilize manual washing.
	Working solutions contain sodium azide	- Avoid adding sodium azide in solutions, as this is a 'peroxidase activity' inhibitor.
Poor standard curve (linearity and dynamic range)	Improper standard dilutions	- Ensure proper dilution of standards (follow 'two-fold dilutions' guidelines).
	Inaccurate pipetting	- Ensure accurate pipetting of volume. - Check pipettes.

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