6459 State Route 30, Lake Clear, NY 12945 518.891.0204

FERRET CYTOKINE PANEL 2 ASSAY PROTOCOL

(Part ID : F107-K; Lot: K23471)

Use

For the detection of 13 ferret cytokines/chemokines **(IFNα, IFNγ, IL-12p40, IL-12p70, IL-17, IL-2, IL-4, IL-6, IL-8, IP-10, MCP-1, MIP-1β, & TNFα**) in serum, plasma, tissue homogenates, and bronchial lavage.

Reagents Supplied

Ferret Cytokine Panel 2 Beads - Part # F107-B
1 vial containing 1.2 mL of beads coupled to capture antibodies for ferret cytokines/chemokines in PBS with 0.1% BSA, 0.02% Tween 20 and 0.05% ProClin 300.

Ferret Cytokine Panel 2 Detection - Part # F107-D
1 vial containing lyophilized biotinylated detection antibodies for ferret cytokines/chemokines in a buffered protein base containing 0.05% ProClin 300.

Ferret Cytokine Panel 2 Standard - Part # F107-S
2 vials containing lyophilized protein standards for ferret cytokines/chemokines with 0.05% ProClin 300.

Ferret Blocker A - Part # F100-BL
1 vial containing lyophilized proprietary mix of domestic animal proteins with 0.05% ProClin 300.

Ferret Standard Diluent A- Part # F100-SCD
1 vial containing lyophilized proprietary mix of domestic animal proteins with 0.05% ProClin 300.

Ferret Cytokine Panel 2 Control 1 - Part # F107-C1
1 vial containing low levels of lyophilized ferret cytokines/chemokines.

Ferret Cytokine Panel 2 Control 2 - Part # F107-C2
1 vial containing high levels of lyophilized ferret cytokines/chemokines.

Streptavidin PE- Part # SAPE4-500
1 vial containing 0.5mL of Streptavidin-Phycoerythrin at 1mg/mL in a solution containing 0.05% ProClin 300.

Sample Diluent - Part # B113-15
1 bottle containing 15 mL of buffered protein base with 0.05% ProClin 300.

10X Assay Buffer - Part # B100-30
1 bottle containing 30 mL of 10X PBS, 0.5% Tween 20 and 0.05% ProClin 300.

Clear Bottom Black Assay Plate

Plate Cover with Foil Sealer

Required Equipment and Materials Not Supplied

- 0.5 mL to 5 mL polypropylene tubes
- Plate washer with magnetic bead separator or manual magnetic bead separator
- Vortex
- Variable volume pipettes and tips (10 µL to 1000 µL)
- Graduated cylinder(s) (50 mL to 1L)
- 1 L bottle
- milliQ H_20 (18.2 MΩ·cm) or equivalent
- Plate shaker (Fisher part #4625Q) or equivalent.
- Luminex®100/200, MAGPIX®, xMAP INTELLIFLEX®, FLEXMAP 3D®, or Bio-Rad® Bio-Plex® system

Storage and Stability

Store all kit components at 2-8°C. Do not use beyond the expiration date.

Protocol

Note: Protocol is designed for assaying one (1) 96-well plate

Equipment and Materials Preparation

1. Set up equipment and materials listed above to be accessible throughout the following protocol.
2. **Luminex Settings**
   Note: Refer to the instrument's manual.
   a. Adjust the probe height on the Luminex appropriately for the plate being used.

   (1) See lot specific certificate of analysis for standard concentrations

Analyte	IFNα	IFNɣ	IL-12p40	IL-12p70	IL-17	IL-2	IL-4
Bead Region	18	12	26	20	27	37	39

Analyte	IL-6	IL-8	IP-10	MCP-1	MIP-1β	TNFα
Bead Region	45	48	54	57	61	62

   (2) Collection parameters
      (a) Sample Volume: 60 µl
      (b) Minimum events: 50 per region
      (c) Flow rate: 60 µL/Min (fast)
      (d) Doublet Discriminator gates: **Use software settings for running MagPlex beads.**
      (e) Collect Median RFU (MFI), low or standard PMT setting is recommended

B. Reagent Preparation:

Note: Reconstitute the entire vial for each of the lyophilized supplied reagents. One time use only for Standards. Blocker, Standard Curve Diluent, Controls, and Detection can be frozen within 2 hours and re-used if needed.

Reconstitute Lyophilized Reagents

a. Reconstitute the following reagents according to the table below.

Reagent	Reconstitute In:	Volume
Standard	milliQ H_20	150 µL
Control 1	milliQ H_20	150 µL
Control 2	milliQ H_20	150 µL
Detection	milliQ H_20	4.2 mL
Blocker	milliQ H_20	1.2 mL
Standard Diluent	milliQ H_20	2.4 mL

b. Vortex at a medium setting.
c. Allow to sit for a minimum of 10 minutes (not to exceed 1 hour).

2. Assay Buffer
   a. Bring the 10X Assay Buffer to room temperature.
   b. Mix by inversion to bring all salts into solution.
   c. Dilute 1 part 10X Assay Buffer with 9 parts of dH_20 (1X Assay Buffer).
   d. Label with preparation date and store at 2-8°C for up to 1 month.
   e. Aliquot approximately 3mL of 1X Assay Buffer for Streptavidin-PE dilution (Step H).

C. Serial Dilution of Standard (1:3.5)

1. Label 8 polypropylene tubes as S1, S2, S3, S4, S5, S6, S7, and S8.
2. Transfer the reconstituted Standard into the tube labeled “S8”.
3. Add the appropriate amount of the Standard Diluent into the labeled tubes according to the table below (this will be sufficient for duplicate standard curves):

Standard	Vol of Std Diluent	Vol of Std
S7	125 µL	50 µL of S8
S6	125 µL	50 µL of S7
S5	125 µL	50 µL of S6
S4	125 µL	50 µL of S5
S3	125 µL	50 µL of S4
S2	125 µL	50 µL of S3
S1	125 µL	50 µL of S2

4. Prepare working Standards at 1:3.5. Serial dilute the appropriate amount of Standard into each of the labeled tubes with Standard Diluent.
5. Vortex at a medium setting and ensure that each Standard is thoroughly mixed.

D. Sample Preparation – Note: Do not dilute controls

1. **Serum/Plasma Samples**
   a. Dilute samples in Sample Diluent 1:3.
   b. For duplicates a suggested dilution of 30µL sample into 60µL Sample Diluent is recommended.
2. **Tissue homogenates**
   a. Dilute samples in 1X Assay Buffer (a suggested concentration for tissue samples is 1000µg/mL).
3. **Bronchial lavage samples**
   a. Bronchial lavage samples can be run neat or diluted 1:2 in 1X Assay Buffer.

E. Add Standard, Controls, Sample, Blocker and Beads to Plate

1. Add 30 µL of the Standard, Control or diluted Sample to the appropriate wells of the plate.
2. Add 10 µL of Blocking Buffer to all wells of the plate.
3. Vortex the Beads at medium speed for 10-20 seconds. Add 10 µL of the Beads to all wells of the plate.
4. Cover plate. Place on plate shaker and incubate at medium-high speed for 10 seconds then decrease speed to medium and **incubate for 2 hours at room temperature in the dark**.

F. Wash Plate

Wash the plate 3X with 200µl of 1X Assay Buffer using either a magnetic bead separator, plate washer or equivalent. (Do not resuspend in buffer)

G. Add Detection to Plate

1. Vortex the Detection at medium speed for 10-20 seconds. Add 40 µL of Detection to all wells of the plate.
2. Cover plate. Place on plate shaker and incubate at medium-high for 10 seconds then decrease speed to medium and **incubate for 1 hour at room temperature in the dark**.

H. Prepare SAPE and Add to Plate

1. Dilute SAPE (1mg/mL) **1:5** in 1X Assay Buffer. For a 96-well plate, dilute 450 µL of SAPE into 1800 µL of 1X Assay Buffer.
2. Add 20 µL of diluted SAPE to all wells of the plate.
3. Cover plate. Place on plate shaker and incubate at medium-high speed for 10 seconds then decrease speed to medium and **incubate for 30 minutes at room temperature in the dark.**

I. Wash Plate

1. Wash the plate 3X with 200µl of 1X Assay Buffer using either a magnetic bead separator, plate washer or equivalent.
2. Resuspend in 100 µl of 1X Assay Buffer.

J. Mix Plate

1. Place plate on plate shaker and mix at medium speed for 1-2 minutes.

K. Read Plate

1. Read the Plate on the Luminex instrument. (Low or standard PMT setting is recommended)

Conditions of Sale

FOR IN VITRO RESEARCH USE ONLY
DO NOT USE FOR DIAGNOSTIC PROCEDURES. DO NOT USE IN HUMANS OR IN ANIMALS