



pbl assay science

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## VeriPlex™ Human Interferon 9-Plex ELISA Kit

Catalog No. 51500

Store **all** components at 2 - 8°C

We recommend reading the protocol in its entirety prior to use. First time users must pay particular attention to pages 12-23 and read the manual of the Q-View™ imager and software available for download at [www.quansysbio.com](http://www.quansysbio.com).

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**51500 Rev. 05**

## A. INTRODUCTION

Interferons (IFNs) are a group of cytokines which exhibit pleiotropic activities that play major roles in both innate and adaptive immunity. There are three types of interferons, namely type I, II and III. Type I IFNs consist of multiple Interferon Alpha (IFN- $\alpha$ ) genes, at least one Interferon Beta (IFN- $\beta$ ) gene, and one Interferon Omega (IFN- $\omega$ ) gene in most vertebrates.<sup>1</sup> IFN- $\alpha$ , IFN- $\beta$  and IFN- $\omega$  are released by a host of mammalian cells on exposure to viruses or double-stranded RNAs,<sup>2</sup> and on triggering of Toll-like receptors (TLR3/4/7/8/9) by CPG DNAs and lipopolysaccharides (LPS). Upon binding to their cellular receptor chains IFN- $\alpha$ -Rc1 and IFN- $\alpha$ -Rc2, Type I IFNs signal through the Jak-Stat pathway to further elicit a host of antiviral actions including production of protein kinase A and 2'5' Oligoadenylate Synthetase (OAS).<sup>2</sup> Type I interferons are used therapeutically to treat viral infections, cancers and autoimmune disorders. IFN- $\alpha$  is used therapeutically to treat hepatitis B and hepatitis C infections. Additionally, IFN- $\alpha$  is known to have significant biological activity in inhibition of proliferation of multiple cancers.<sup>3</sup> IFN- $\beta$  is used therapeutically to treat multiple sclerosis.<sup>4</sup> Type II IFN consists of Interferon Gamma (IFN- $\gamma$ ). IFN- $\gamma$  is produced by a host of immune cells - lymphocytes, CD4+ T cells, NK cells and such antigen presenting cells (APCs) as macrophages, monocytes and dendritic cells.<sup>5</sup> IFN- $\gamma$  uses receptor chains IFN- $\gamma$ -R1 and IFN- $\gamma$ -R2. IFN- $\gamma$ , a homodimer, binds two IFN- $\gamma$ -R1 subunits, thereby generating binding sites for two IFN- $\gamma$ -R2 chains, a process that subsequently triggers intracellular signaling and activation of Jak1, Jak2 and Stat1 that in turn induce genes with the  $\gamma$  activation sequence in the promoter.<sup>2,6</sup> IFN- $\gamma$  plays a role in several immunomodulatory functions including up-regulation of pathogen recognition, antiviral action, activation of microbicidal functions in immune cells and leukocyte trafficking.<sup>5</sup> The newly

characterized Type III IFNs consist of Interferon Lambda 1 (IFN- $\lambda$ 1 or IL-29), Interferon Lambda 2 (IFN- $\lambda$ 2 or IL-28A) and Interferon Lambda 3 (IFN- $\lambda$ 3 or IL-28B). The members of the Type III family share close homology to one another with IFN- $\lambda$ 2 and IFN- $\lambda$ 3 sharing 96% amino acid identity and with IFN- $\lambda$ 1 sharing 81% homology with IFN- $\lambda$ 2 and IFN- $\lambda$ 3.<sup>7</sup> Type III IFNs are functionally similar to Type I IFNs and are known to have similar downstream effects, i.e. Type III IFNs promote the phosphorylation on STAT1 and STAT2, induce the ISRE3 complex, elevate OAS and Type I IFNs induce Myxovirus resistance protein A (MxA) expression and exhibit antiviral activity *in vitro*;<sup>10</sup> however, Type III IFNs and their heterodimeric receptor subunits-CRF2-12(IFN- $\lambda$ R1)<sup>9</sup> and CRF2-4 (IL-10R2)<sup>9</sup> are known to be more prominent in cells of the epithelial tissues.<sup>8</sup>

IFN- $\lambda$ 1 is known to modulate the development of Th1/Th2 cells.<sup>10</sup> IFN- $\lambda$ 2 has been shown to inhibit the replication of hepatitis B and hepatitis C virus in murine hepatocyte cell lines<sup>11</sup> and IFN- $\lambda$ 1 is being explored as a potential therapeutic for hepatitis C.<sup>12</sup>

The VeriPlex™ Human Interferon 9-Plex ELISA has been developed to simultaneously detect IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , IFN- $\omega$ , IFN- $\lambda$ 1/2/3 and other key pro-inflammatory cytokines released upstream and downstream of interferon signaling, including TNF- $\alpha$ , IP-10, IL-1 $\alpha$  and IL-6. This assay has been developed using the Q-Plex™ array spotting technology, in which capture antibodies to the different analytes are spotted in a single well in a 3x3 array. The functional format of the assay is as that of a sandwich ELISA with a chemiluminiscent output. The assay is compatible with multiple matrices including tissue culture media, human serum, human plasma and buffers.



## **MATERIALS PROVIDED**

- Human Interferon Multiplex 96-Well microtiter plate
- Plate sealers
- Wash Solution Concentrate
- Human Interferon Multiplex Antigen Standard
- Sample Diluent
- Assay Diluent
- Human Interferon Multiplex Detection Mix
- HRP Concentrate
- Substrate A
- Substrate B
- Diluent Additive II
- Diluent Additive III

## **ADDITIONAL MATERIALS REQUIRED (NOT PROVIDED)**

- Variable volume microtiter pipettes
- Adjustable multichannel pipette (50-300  $\mu$ l)
- Reagent reservoirs
- Wash bottle or plate washing system
- Distilled or deionized water
- Serological pipettes (1, 5, 10 or 25 ml)
- Disposable pipette tips (polypropylene)
- Plate shaker
- One of the following cameras/imagers:
  - Q-View™ Imager (recommended)
  - Alpha Innotech HD2 and FC2 Camera
  - Bio-Rad VERSADOC 4000 Camera
  - Bio-Rad CHEMIDOC XRS Camera
  - Fujifilm LAS 3000 Camera
  - KODAK 4000MM Camera
- Q-View™ software

*Specifications:* This kit quantitates Human Interferon Alpha (IFN- $\alpha$ ), Human Interferon Beta (IFN- $\beta$ ), Human Interferon Gamma (IFN- $\gamma$ ), Human Interferon Omega (IFN- $\omega$ ), Human Interferon Lambda (IFN- $\lambda$  1, 2 and 3), Human Tumor Necrosis Factor-Alpha (TNF- $\alpha$ ), Human Interleukin-6 (IL-6), Human Interferon Gamma inducible protein (IP-10) and Human Interleukin 1 Alpha (IL-1 $\alpha$ ) in sera, plasma and tissue culture media by sandwich enzyme linked immunosorbent assay (ELISA) using the Q-Plex™ Multiplex technology.

*Detection Ranges:* Refer to the supplied lot specific Certificate of Analysis

*Speed:* Incubation time, 3 hr 15 min

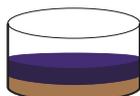
*Specificity:* The IFN- $\lambda$  antigen in the Standard is an equal mix of IFN- $\lambda$ 1 and  $\lambda$ 2. The IFN- $\lambda$  antibody pair in the product detects IFN- $\lambda$ 1,  $\lambda$ 2 and  $\lambda$ 3, although IFN- $\lambda$ 2 and  $\lambda$ 3 are detected less effectively.

Rhesus monkey IFN- $\alpha$  A, Cynomolgus monkey IFN- $\alpha$ , and Cynomolgus monkey IFN- $\beta$  are detected by the product. Mouse IFN- $\beta$ , Mouse IFN- $\gamma$  and Mouse IFN- $\lambda$ 3 do not cross react with the product. 10  $\mu$ g/ml of Mouse IFN- $\alpha$  demonstrated 0.004% cross reactivity. Refer to pages 29-31 for details.

*Storage Conditions/Comments:* For retention of full activity, all reagents should be kept at 2-8°C in the dark when not in use. Diluents and buffer reagents should be warmed to room temperature (RT), 22-25°C, before use. We have not fully evaluated the long term stability of reconstituted materials in liquid or frozen form.

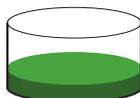
## B. ASSAY PROCEDURE - QUICK REFERENCE

**Total Time:** 3 hr, 15 min



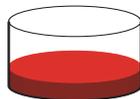
- 1) Add **50  $\mu$ l** Assay Diluent
- 2) Add **50  $\mu$ l** Standard, Test Sample or Blank

Incubate **2 hrs** (shake at 500 rpm)  
Aspirate and wash **3x** (30 second soak between washes)



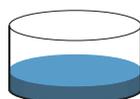
Add **50  $\mu$ l** Reconstituted Detection Mix

Incubate **1 hr** (shake at 500 rpm)  
Aspirate and wash **3x** (30 second soak between washes)



Add **50  $\mu$ l** HRP Concentrate

Incubate **15 min** in the dark (shake at 500 rpm)  
Aspirate and wash **6x** (30 second soak between washes)



Add **50  $\mu$ l** Mix of Substrate A and B  
Image plate within 20 min

**Note:** ALL incubations are at room temperature (22 - 25°C)

### C. PREPARATION OF REAGENTS

Supplied Human IFN Multiplex Antigen Standard, Human IFN Multiplex Detection Mix and HRP Concentrate should be kept on wet ice.

**Wash Solution:** The Wash Solution Concentrate may contain crystals. Place the bottle in a warm water bath and gently mix until completely dissolved. Prepare a 1:20 working wash solution (e.g. Add 50 ml of Wash Solution Concentrate to 950 ml of distilled or deionized water and mix thoroughly). Diluted Wash Solution can be stored at RT (22-25°C) when not in use.

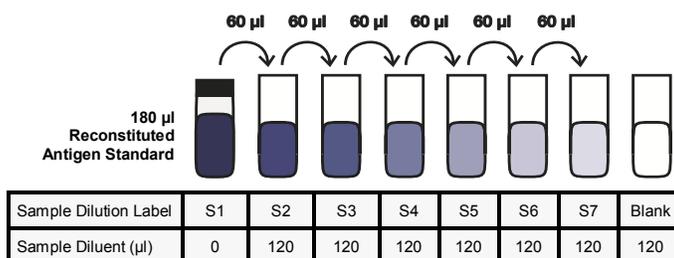
**Standard:** Reconstitute the supplied Human IFN Multiplex Antigen Standard by adding the volume of Sample Diluent indicated in the lot specific Certificate of Analysis. Mix gently until the Antigen Mix is completely dissolved and store on wet ice until use. Do not vortex. Do not introduce bubbles.

**Standard Curve Preparation:** Label seven polypropylene tubes (S1-S7). Prepare a 3 fold dilution series using the reconstituted Antigen Standard and Sample Diluent as per Figure 1. Mix thoroughly between each dilution by pipeting 5x. The high point (S1) in the series is the reconstituted Antigen Standard.

**Assay Diluent:** Reconstitute the Assay Diluent as per instructions in the lot specific Certificate of Analysis (COA) using distilled or deionized water, Diluent Additive II and Diluent Additive III. Do not vortex. Store at RT (22-25°C) until use in step 1 of the Assay Procedure.

**Note:** Solution may appear cloudy and contain bubbles.

**Figure 1: 7-Point Standard Curve Prepared in Sample Diluent**



**Detection Mix:** Reconstitute the Human IFN Multiplex Detection Mix in 6 ml distilled or deionized water. Mix gently until the mix is completely reconstituted. Store on ice until use in step 2 of the Assay Procedure.

#### D. ASSAY PROCEDURE

All incubations should be performed in a closed chamber at RT (22-25°C), keeping the plate away from drafts and other temperature fluctuations. Use a plate shaker at 500 rpm where indicated. The shaking speed must be such that bubbles are not formed during shaking. Use plate sealers to cover the plates as directed. During all wash steps, remove contents of plate by inverting and shaking over a sink and blotting the plate on lint-free absorbent paper; tap the plate. **It is recommended that the plate be soaked in Wash Solution for 30 seconds between washes.** Wash each well with a minimum of 300 µl of diluted Wash Solution for each wash step. See Preparation of Reagents for details on dilution of concentrated solutions.

1. **Standards and Test Samples:**

1a. **Adding Assay Diluent:**

- Add 50  $\mu$ l of Assay Diluent to each well

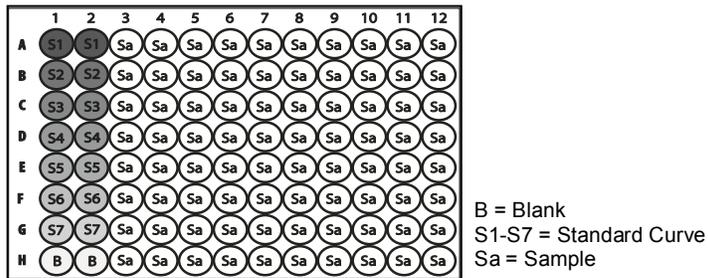
We recommend running the standard, blanks and test samples in duplicate. Refer to the model plate setup in Figure 2. **The highest dilution point of the standard (S1) must be in wells A1 and A2.**

1b. **Adding Standards, Blanks and Test Samples:**

- Add 50  $\mu$ l of Standard to wells designated as Standard
- Add 50  $\mu$ l of Blanks (Sample Diluent or sample matrix) to wells designated as Blanks
- Add 50  $\mu$ l Test Samples to wells designated as Test Samples

Cover with plate sealer and shake plate at 500 rpm at RT (22-25°C) for 2 hours.

**Figure 2: Example of a Typical Plate Setup**



After 2 hours, empty the contents of the plate and wash the wells three times with at least 300 µl of working Wash Solution (refer to Preparation of Reagents) per well (30 second soak recommended between washes).

2. **Detection Antibody:** Add 50 µl of reconstituted Detection Mix (refer to Preparation of Reagents) to each well. Cover with plate sealer and shake plate at 500 rpm at RT (22-25°C) for 1 hour.

**During this incubation, prepare a Substrate Mix by mixing equal volumes of Substrate A and Substrate B. Use full contents of Substrate A and Substrate B. Store the mix at RT (22-25°C), in the dark, until use in Step 4. The mix must be prepared at least 10 minutes prior to use in Step 4.**

After 1 hour, empty the contents of the plate and wash the wells three times with at least 300 µl of working Wash Solution per well (30 second soak recommended between washes).

3. **HRP Concentrate:** Add 50 µl of supplied HRP Concentrate to each well. Cover with plate sealer and shake, in the dark, at 500 rpm at RT (22-25°C) for 15 minutes.

After 15 minutes, empty the contents of the plate and wash the wells six times with at least 300 µl of working Wash Solution per well (30 second soak recommended between washes).

4. **Substrate Mix and Imaging:** Add 50 µl of the prepared Substrate Mix to each well.

**Image the plate within 20 minutes of adding the Substrate Mix. Refer to pg. 12 and onwards for detailed instructions on imaging the plate using different imagers.**

## E. IMAGING PROCEDURE

### 1. Quansys Q-View™ Imager—Acquiring an Image

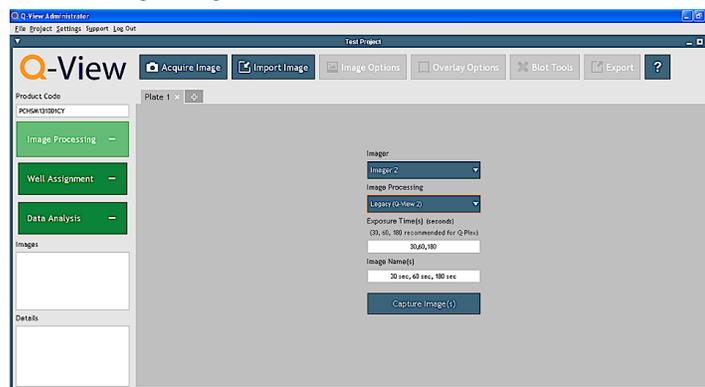
These are basic instructions for using the Q-View™ Imager and Software to image your plate only. A comprehensive software manual for use of Q-View™ software is available.

A full version of the Quansys Q-View™ Software is available for free. The download is available at <http://www.quansysbio.com/q-view-software>.

The user manual for the software can be found under Manuals in the Support section.

- A. Select New Project if starting a new project and save under a new name. Otherwise, select Open Project to browse and select a previous project. The new image will not overwrite prior images in the project.
- B. Ensure that the Q-View™ Imager is connected to the computer. The connection status can be confirmed under Settings -> Administration -> Manage Imagers. If needed, click on Refresh.
- C. *Optional.* Uncheck the box “Discard sub-images after stacking is complete” under Settings -> Preferences in order to see images of different exposure times after the imaging process is complete. Otherwise, a stacked image will be displayed.
- D. *Optional.* It is recommended to periodically calibrate the Q-View™ Imager. To calibrate the imager, select Settings -> Administration -> Manage Imagers. Ensure that there is no plate in the plate housing slot in the imager and that the housing door is closed. Select Calibrate.
- E. *Optional.* If the imager has not been focused previously, place a Quansys focusing plate in the imager (do not close

the plate housing door) and adjust the focus (under Settings -> Administration -> Manage Imagers) until the image is clear. Remove the focusing plate. Close the Manage Imagers section.



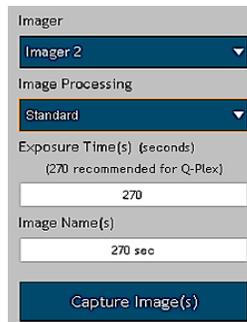
Q-View™ Version 3.01

- F. Select Acquire Image.
- G. Ensure that the Q-View™ Imager is recognized. If not, follow step B. and click on Refresh.
- H. Select Image Processing method, either Legacy (similar to previous versions of Q-View™) or Standard (new feature in Q-View™).
- I. Enter Exposure Time(s). Recommended exposure times are 30, 60 and 180 seconds for the Legacy setting or 270 seconds for Standard Image Processing. When using Legacy, each exposure will have a different image. The software will also display a stacked image.
- J. Enter the names of the image(s). For example, Expt1 30 sec, Expt1 60 sec and Expt1 180 sec.
- K. Place the plate to be read in the plate housing slot, close

the plate housing door and select Capture Image(s). The imaging should begin. Once acquired, the image will appear in the Q-View™ Software main screen.

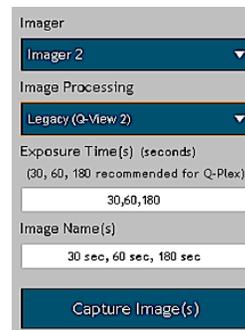
- L. If the image is needed for use in other programs, save the acquired image(s) by clicking Export. Export the image(s) as TIFF file(s).

Exposure Times: These times can be modified to meet your specific assay, but 30, 60 and 180 second exposure times (Legacy) or 270 second exposure time (Standard) are recommended for most assays. Once set, one can exit the Exposure Times dialog by mousing outside of the dialog box.



The dialog box for the Standard Image Acquisition Option features a grey background. At the top, it is labeled 'Imager' with a dropdown menu set to 'Imager 2'. Below this is the 'Image Processing' section with a dropdown menu set to 'Standard'. The 'Exposure Time(s) (seconds)' field is set to '270', with a note '(270 recommended for Q-Plex)' above it. The 'Image Name(s)' field contains '270 sec'. At the bottom is a blue 'Capture Image(s)' button.

Standard Image Acquisition Option



The dialog box for the Legacy Image Acquisition Option features a grey background. At the top, it is labeled 'Imager' with a dropdown menu set to 'Imager 2'. Below this is the 'Image Processing' section with a dropdown menu set to 'Legacy (Q-View 2)'. The 'Exposure Time(s) (seconds)' field is set to '30,60,180', with a note '(30, 60, 180 recommended for Q-Plex)' above it. The 'Image Name(s)' field contains '30 sec, 60 sec, 180 sec'. At the bottom is a blue 'Capture Image(s)' button.

Legacy Image Acquisition Option

## 2. Q-View™ Software—Importing an Image File

Q-View™ Software can open images in the following formats: TIFF, CR2 (raw image files from Canon cameras), JPEG, PNG and BMP. However, it should be noted that lossy or low bit depth images (JPEG, PNG and BMP) are insufficient to use for analysis and therefore should be imported for the purpose of

display only. Users should take images using supported imaging systems (See Page 5).

To acquire an image by importing an image file, select Import Image. Browse and select the desired image.

The time to upload the image will vary depending on the image file type and size. Once imported, the image will appear in the Q-View™ Software main screen.

*Images acquired using the following imagers can be imported into the Q-View™ software for analysis. Refer above for details on importing images to Q-View™ software.*

### 3. Acquiring an image using the Alpha Innotech HD2 Camera and Software Setup

- A. Open the camera door.
- B. Set the adjustable tray on the lowest level.
- C. Place the focusing plate in the center of the tray for focusing.
- D. Open the aperture on the camera all the way to the lowest value (~0.95).
- E. Open the software on the computer.
- F. Click the acquire button.
- G. Close the door on the camera slightly so some light can get in and the focusing plate can be seen on the computer screen.
- H. Adjust the focus on the actual camera lens until the focusing plate is in focus.
- I. Remove the focusing plate.
- J. Place the plate to be read in the center of the tray and make sure it is in the center of the photo path (on the computer screen).

- K. Close all doors on the camera and ensure there are no light leaks.
- L. Ensure all cabinet lights are off and that the filter wheel is set to position "1."
- M. Set the software settings on the computer as follows:
  - i. No lights on.
  - ii. Resolution to "Normal/Ultra".
  - iii. Select only "noise reduction"
  - iv. Set the exposure time to 3 min for the first time.
- N. Click on Acquire Image.
- O. Once the image is acquired, save it and look at the pixel intensity of the high points on the standard curve. On average, most of the high points on the curve should be in the 45,000 - 55,000 pixel intensity range, and on the second spot they should be in the 20,000 - 40,000 pixel intensity range.
  - i. If there are spots where the PI (pixel intensity) on the high point of the curve is at 65,000 and the second point is 60,000 or higher, then acquire another image of the plate for a shorter period of time.
  - ii. If the spots on the high point of the curve in general fall below 40,000 PI, then reacquire the image for a longer period of time.

#### 4. Acquiring an image using the Alpha Innotech FC2 Camera and Software Setup

- A. Open the camera door.
- B. Set the adjustable tray on the top shelf.
- C. Place the focusing plate in the center of the tray for focusing.
- D. Open the aperture on the camera all the way to the lowest value (~1.8).

- E. Open the software on the computer.
- F. Click the Acquire button.
- G. Close the door on the camera slightly so some light can get in and the focusing plate can be seen on the computer screen.
- H. Adjust the focus on the actual camera lens until the focusing plate is in focus.
- I. Remove the focusing plate.
- J. Place the plate to be read in the center of the tray and make sure it is in the center of the photo path (on the computer screen).
- K. Close all doors on the camera and ensure there are no light leaks.
- L. Ensure all cabinet lights are turned off and that the filter wheel is set to "1".
- M. Set the software settings on the computer.
  - i. No lights on.
  - ii. Resolution to "Normal/Ultra".
  - iii. Select only "noise reduction",
  - iv. Set the exposure time to 6 min for the first time.
- N. Click on Acquire Image.
- O. Once the image is acquired, save it and look at the pixel intensity of the high points on the standard curve. On average, most of the high points on the curve should be in the 45,000 - 50,000 pixel intensity range, and on the second spot they should be in the 20,000 - 40,000 pixel intensity range.
  - i. If there are spots where the PI (pixel intensity) on the high point of the curve is at 65,000 and the second point is 60,000 or higher, then acquire another image of the plate for a shorter period of time.
  - ii. If the spots on the high point of the curve in general fall below 40,000 PI, then reacquire the image for a longer period of time.

5. Acquiring an image using the Bio-Rad VERSADOC 4000 Camera and Software Setup

A. File setup

- i. Open the software on the computer.
- ii. Click on "File, then select "Versadoc".
- iii. Make sure that only "Channel 1" is enabled.
- iv. Click on the "Select" button, then select "Custom" and then "Create".
- v. When the new window opens, name this custom setup as "Quansys 1x1 binning", and change the settings to:
  1. Filter: None
  2. Illumination: None
  3. Gain: 1X
  4. Binning: 1X1
- vi. Click on "OK". (Now that this setting is saved, you can use it again for future exposures. Instead of selecting "Create", select "Quansys 1X1 binning".)

B. Camera Setup and Focus

- i. Open the imager door.
- ii. Place a box or stand in the cabinet below the camera to increase the imaging height between 4 - 6 inches.
- iii. Place the "imaging target sheet" or the focusing plate in the cabinet on top of the box or stand.
- iv. Open the aperture on the camera all the way to the lowest value.
- v. Leave the door slightly open to let in light while focusing.
- vi. Click "Focus" in the software, and turn the focus on the camera until the imaging targets on the screen on the computer are in focus or the focusing plate is in focus. Click "Stop" when in focus.
- vii. Replace the "imaging target sheet"/focusing plate with the plate to be read, and ensure the plate is centered

- in the imaging screen and is straight.
- viii. Close the cabinet door.
- C. Image Acquisition
- i. On “Step III—Set exposure time” on the software, change the exposure time to 30 seconds and select Acquire.
  - ii. When the exposure is complete, convert the image into negative (black background with white spots).
    1. Click image, and new menu appears.
    2. Select Transform, and then check the box that says “Invert Display”.
    3. Click OK.
  - iii. Save the image.
- D. Image Optimization
- i. Once the image is acquired, look at the pixel intensity of the high points on the second dilution in the standard curve, and make sure they are not saturated. On average, most of the high points on the curve should be in the 45,000 - 55,000 Pixel Intensity range, and on the second spot they should be in the 20,000 - 40,000 Pixel Intensity range.
    1. If there are spots where the PI (pixel intensity) on the high point of the curve is at 65,000 and the second point is 60,000 or higher, then re-expose image of the plate for a shorter period of time (1 minute).
    2. If the spots on the high point of the curve in general fall below 40,000 PI, then re-expose the image for a longer period of time (i.e. 3 minutes).
- E. File Conversion
- i. After acquiring the images, you need to convert them to TIFF files.
  - ii. Click on “File” then “Export to TIFF image”.
  - iii. Select “Export raw data”, click on “Export”, then click on “Save”.

6. Acquiring an image using the Bio-Rad CHEMIDOC XRS Camera and Software Setup

A. File Setup

- i. Open the software on the computer.
- ii. Click on "File", then select "ChemiDoc XRS".
- iii. Under "Step I—Select Application" press the "Select" button, then select "Custom" and then "Create".
- iv. When the new window opens, name this custom setup as "Quansys 1x1 binning". Under "Illumination" select "none", and under "gain & binning" select "2X, 1X1" and click "OK". (Now that this setting is saved you can use it again for future exposures. Instead of selecting "Create", select "Quansys 1x1 binning").

B. Camera Setup and Focus

- a. On the software select "Live Focus".
- b. On the cabinet press the plus button to open the aperture or iris all the way (the lowest number).
- c. Open the camera's drawer, place the plate in the middle of the drawer, then close the drawer.
- d. Press the "Epi White" button on the camera cabinet.
- e. Press the zoom buttons on the cabinet until the plate fills most of the screen on the computer.
- f. Replace the plate with the "imaging target sheet" or the focusing plate and close the door.
- g. Press the focus buttons on the cabinet until the targets or focusing plate are in focus on the computer screen.
- h. Replace the imaging target sheet/focusing plate with the plate to be read, close the drawer, and make sure the plate is in the center of the imaging screen and straight.
- i. Turn off the "Epi White" button on the cabinet, and select "freeze" in the software.

C. Image Acquisition

- a. On "Step III—Acquire Image" on the software, change the exposure time to 30 seconds and select Manual Expose.
- b. When the exposure is complete, convert the image into negative (black background with white spots).
  - i. Click Image, and new menu appears.
  - ii. Select Transform, and then check the box that says "Invert Display".
  - iii. Click OK.
- c. Save the image.

D. Image Optimization

- a. Once the image is acquired, look at the pixel intensity of the high points on the standard curve. On average, most of the high points on the curve should be in the 45,000 - 55,000 Pixel Intensity range, and on the second spot they should be in the 20,000 - 40,000 Pixel Intensity range.
  - i. If there are spots where the PI (pixel intensity) on the high point of the curve is at 65,000 and the second point is 60,000 or higher, then re-expose image of the plate for a shorter period of time (i.e. 1 minute).
  - ii. If the spots on the high point of the curve in general fall below 40,000 PI, then re-expose the image for a longer period of time (i.e. 3 minutes).

E. File Conversion

- a. After acquiring the images, you need to convert them to TIFF files.
- b. Click on "File" then "Export to TIFF image".
- c. Select "Export raw data", click on "Export", then click on "Save".

## 7. Acquiring an image using the Fujifilm LAS-3000 Camera and Software Setup

### A. File Setup

- a. Open the software on the computer.
- b. Under "Exposure Type" select "Precision" in the drop down menu.
- c. Under "Exposure Time" set the imager to take a 30-second image by selecting "Manual" then entering "30" in the first box and selecting "sec" in the second box.
- d. Under "Sensitivity" select "Standard" in the drop down menu.
- e. Ensure the box next to "Image Acquire & Digitize" is checked.
- f. Click on the "Method/Tray Position" button. In the window that appears, select "Chemiluminescence", and under tray position select "2". Then select "OK" to close the window.

### B. Camera Setup and Focus

- a. Open the camera box and make sure the tray is in position "2".
- b. Place the focusing plate or other imaging target sheet on the tray and close the door.
- c. On the software select "Focus" and a new window appears. In the "Adjust" area, click up or down on the arrows until the focusing plate is in focus.
- d. Remove the focusing plate from the imager and place the plate to be read in the center of the tray. Look on the computer screen to make sure the plate is centered and straight in the imaging screen. Close the imager door when the plate is centered.
- e. Select the "Return" button on the software to close the focusing window.

C. Image Acquisition

- a. When the plate is ready to image, press the “Start” button.
- b. After the plate has imaged invert the image to black with white spots by clicking on “View” then selecting “Positive Gray” in the drop down menu.
- c. Save the image by pressing the “Save” button. In the new window select “16 bit linear TIFF” in the “Save as type” drop down menu. Then type a name for the file and select “Save”.
- d. Press the “Complete” button to allow the imager to take another image.
- e. Take multiple images at different exposure times to ensure you get the best reading possible. Example exposure times are 20 seconds, 45 seconds, 60 seconds, 90 seconds and 120 seconds.

D. Image Optimization

- a. Once the image is acquired, look at the pixel intensity of the high points on the standard curve. On average, most of the high points on the curve should be in the 45,000 - 55,000 Pixel Intensity range, and on the second spot they should be in the 20,000 - 40,000 Pixel Intensity range.
  - i. If there are spots where the PI (pixel intensity) on the high point of the curve is at 65,000 and the second point is 60,000 or higher, then re-expose image of the plate for a shorter period of time (i.e. 20 seconds).
  - ii. If the spots on the high point of the curve in general fall below 40,000 PI, then re-expose the image for a longer period of time (i.e. 2 minutes).

## F. PRODUCT PERFORMANCE CHARACTERIZATION

### 1. Matrix Studies:

#### i. Levels of analytes in Normal human serum and Normal human plasma

Normal human serum from 20 individual donors and Normal human plasma with different anti-coagulants (Na-EDTA, Na-Citrate, and Na-Heparin) from 13 other individual donors were tested in the assay. The levels of analytes in the samples were extrapolated from a Standard Curve prepared in Sample Diluent.

Serum and plasma from all donors had detectable levels of IP-10. The average concentration of IP-10 in serum was 55.6 pg/ml, while in plasma was 86.9 pg/ml.

Serum from one donor had 16.0 pg/ml of IFN- $\alpha$ , serum from a second donor had 6.4 pg/ml of IL-1  $\alpha$  and 40 pg/ml of IL-6, serum from a third donor had 9.1 pg/ml of IL-1  $\alpha$ , 1935.2 pg/ml of IL-6, and 27.0 pg/ml of TNF- $\alpha$ , and serum from a fourth donor had 43.4 pg/ml of IL-6.

Serum from remaining 16 donors had undetectable levels of analytes other than IP-10.

Plasma from all 13 donors had undetectable levels of analytes other than IP-10.

Analyte	Normal human serum (20 donors)		Normal human plasma (13 donors)	
	Average (pg/ml)	Range (pg/ml)	Average (pg/ml)	Range (pg/ml)
IFN- $\alpha$	<LLOD	<LLOD to 16.0	<LLOD	N/A
IFN- $\beta$	<LLOD	N/A	<LLOD	N/A
IFN- $\gamma$	<LLOD	N/A	<LLOD	N/A
IFN- $\lambda$ 1/2	<LLOD	N/A	<LLOD	N/A
IFN- $\omega$	<LLOD	N/A	<LLOD	N/A
IL-1 $\alpha$	<LLOD	<LLOD to 9.1	<LLOD	N/A
IL-6	102.7 *	<LLOD to 1935.2	<LLOD	N/A
IP-10	55.6	9.14 to 130.3	86.9	21.3 to 182.2
TNF- $\alpha$	<LLOD	<LLOD to 27.0	<LLOD	N/A

\* Average value of IL-6 in normal human serum was high due to presence of 1935.2 pg/ml of IL-6 in serum from a particular donor. Levels in serum from 17 donors were either close to LLOD or < LLOD.

N/A-Not applicable because levels in serum or in plasma from all donors were < LLOD.

ii. Spike Recovery

Low, medium, and high spikes were prepared using the Multiplex Antigen Standard in Normal human serum from a single donor, Normal human plasma with different anticoagulants, TCM-DMEM+10% FBS, and Sample Diluent. The concentration of spikes were extrapolated from a Standard Curve prepared in Sample Diluent. The recoveries in Normal human serum and in Normal human plasmas were calculated after subtracting measured levels of endogenous analytes, in the matrices, from the recovered values.

a. High Spike

Analyte	Sample Diluent	DMEM+10% FBS	Normal human serum (donor A)	Normal human plasma with Na-EDTA (donor B)	Normal human plasma with Na-EDTA (donor C)	Normal human plasma with Na-Heparin (donor C)	Normal human plasma with Na-Citrate (donor D)
IFN- $\alpha$	82.8%	105.3%	91.6%	38.0%	42.7%	114.4%	86.5%
IFN- $\beta$	79.6%	77.8%	125.8%	87.2%	110.0%	108.1%	102.7%
IFN- $\gamma$	113.8%	88.0%	98.8%	95.5%	92.5%	100.7%	92.7%
IFN- $\lambda$ 1/2	96.5%	95.5%	94.0%	89.9%	94.0%	101.3%	83.3%
IFN- $\omega$	82.9%	77.3%	82.5%	76.8%	104.8%	104.2%	86.7%
IL-1 $\alpha$ *	92.4%	85.0%	76.9%	0.1%	79.7%	71.6%	63.2%
IL-6	81.8%	75.6%	69.1%	50.0%	77.1%	79.1%	68.4%
IP-10	87.2%	77.7%	83.8%	73.5%	64.6%	83.6%	75.0%
TNF- $\alpha$	91.4%	72.6%	81.3%	79.2%	87.0%	88.5%	60.6%

\* The recovery of high, medium and low IL-1 $\alpha$  spikes in normal human plasma with Na-EDTA from donor B was particularly poor.

b. Medium Spike

Analyte	Sample Diluent	DMEM+ 10%FBS	Normal human serum (donor A)	Normal human plasma with Na-EDTA (donor B)	Normal human plasma with Na-EDTA (donor C)	Normal human plasma with Na-Heparin (donor C)	Normal human plasma with Na-Citrate (donor D)
IFN- $\alpha$	100.0%	121.8%	107.1%	33.0%	43.0%	106.5%	73.3%
IFN- $\beta$	82.6%	97.9%	138.4%	103.3%	98.7%	100.2%	98.1%
IFN- $\gamma$	125.5%	97.5%	110.6%	104.9%	89.4%	94.4%	77.3%
IFN- $\lambda$ 1/2	114.9%	118.0%	115.5%	104.2%	84.2%	87.7%	72.6%
IFN- $\omega$	97.2%	87.5%	101.1%	95.7%	105.8%	109.6%	101.4%
IL-1 $\alpha$ *	102.3%	89.9%	80.6%	0.0%	59.5%	55.6%	47.9%
IL-6	98.2%	84.5%	79.1%	57.2%	75.8%	71.7%	61.3%
IP-10	97.7%	81.0%	96.1%	77.4%	63.4%	75.6%	50.8%
TNF- $\alpha$	99.3%	77.5%	86.2%	77.8%	85.1%	88.2%	57.1%

c. Low Spike

Analyte	Sample Diluent	DMEM+ 10%FBS	Normal human serum (donor A)	Normal human plasma with Na-EDTA (donor B)	Normal human plasma with Na-EDTA (donor C)	Normal human plasma with Na-Heparin (donor C)	Normal human plasma with Na-Citrate (donor D)
IFN- $\alpha$	91.5%	124.2%	109.0%	23.9%	35.5%	91.2%	81.0%
IFN- $\beta$	69.8%	94.2%	161.8%	111.3%	95.2%	87.4%	98.3%
IFN- $\gamma$	133.9%	85.1%	113.3%	108.4%	44.7%	72.7%	63.1%
IFN- $\lambda$ 1/2	93.2%	94.2%	75.5%	91.1%	47.4%	56.1%	54.4%
IFN- $\omega$	90.8%	82.1%	106.0%	89.2%	62.1%	78.7%	79.5%
IL-1 $\alpha$ *	102.2%	73.2%	64.1%	0.0%	25.3%	20.6%	9.0%
IL-6	95.4%	82.0%	78.4%	67.3%	65.0%	54.2%	49.9%
IP-10 ++	117.2%	67.8%	113.9%	79.6%	-16.5%	-9.4%	-70.8%
TNF- $\alpha$	102.1%	69.4%	86.0%	75.6%	66.9%	60.9%	41.9%

++ Poor recoveries of low IP-10 spikes in plasma are due to presence of ~110 pg/ml of apparent endogenous IP-10 in the plasma lots.

iii. Intra-assay and Inter-assay % CV

a. Intra-assay % CV

Analyte	Sample Diluent	Normal human serum (donor A)	DMEM+ 10%FBS	Normal human plasma with Na-EDTA (donor B)
IFN- $\alpha$	4.3%	3.9%	4.6%	7.0%
IFN- $\beta$	4.3%	8.2%	3.8%	8.0%
IFN- $\gamma$	6.3%	3.8%	6.5%	6.5%
IFN- $\lambda$ 1/2	7.0%	7.7%	11.4%	9.2%
IFN- $\omega$	4.6%	7.0%	5.5%	6.5%
IL-1 $\alpha$	4.9%	6.3%	5.6%	15.7%
IL-6	7.2%	5.1%	7.4%	2.1%
IP-10	3.4%	8.5%	3.0%	3.3%
TNF- $\alpha$	4.4%	4.3%	4.6%	3.9%

b. Inter-assay % CV

Analyte	Sample Diluent	Normal human serum (donor A)	DMEM+ 10%FBS	Normal human plasma with Na-EDTA (donor B)
IFN- $\alpha$	8.6%	3.8%	7.6%	15.5%
IFN- $\beta$	9.8%	13.9%	12.1%	12.6%
IFN- $\gamma$	8.9%	8.9%	14.3%	15.6%
IFN- $\lambda$ 1/2	14.1%	12.4%	20.2%	23.9%
IFN- $\omega$	6.2%	7.3%	11.0%	8.0%
IL-1 $\alpha$	9.2%	9.8%	14.9%	ND
IL-6	12.8%	19.0%	19.8%	25.1%
IP-10	8.4%	10.0%	13.9%	21.5%
TNF- $\alpha$	13.2%	12.4%	12.9%	12.7%

## 2. Cross Reactivity Studies

### i. IFN- $\lambda$ subtypes:

Independent curves of recombinant human IFN- $\lambda$ 1, recombinant human IFN- $\lambda$ 2, and recombinant human IFN- $\lambda$ 3 were prepared in Sample Diluent. A separate standard curve was prepared using the Human IFN Multiplex Antigen Standard supplied in the product. The % recovery of those points with pixel intensities within the range of pixel intensities of IFN- $\lambda$ 1/2 in the multiplex standard were averaged to estimate the % Reactivity for each subtype.

<b>Subtypes</b>	<b>% Reactivity</b>
IFN- $\lambda$ 1	167.4%
IFN- $\lambda$ 2	39.5%
IFN- $\lambda$ 3	41.7%

### ii. Human IFN- $\alpha$ subtypes, Rhesus Monkey IFN- $\alpha$ , Cynomolgus Monkey IFN- $\alpha$ (Ile16) and Cynomolgus Monkey IFN- $\beta$

Independent curves of recombinant analytes listed in the table on the next page were prepared in Sample Diluent. A separate standard curve was prepared using the Human IFN Multiplex Antigen Standard supplied in the product. The % recovery of those points on the curves of the test analytes with pixel intensities within the range of pixel intensities of IFN- $\alpha$  A 2a in the curve prepared using the Multiplex Antigen Standard were averaged to estimate the % Reactivity for each analyte.

Catalog No.	Analyte	% Reactivity
11105-1	Human IFN- $\alpha$ 2 ( $\alpha$ 2b)	66.7%
11100-1	Human IFN- $\alpha$ A ( $\alpha$ 2a)	55.7%
11175-1	Human IFN- $\alpha$ 1 { $\alpha$ D (Ala 114)}	30.6%
11165-1	Human IFN- $\alpha$ K ( $\alpha$ 6)	20.0%
11120-1	Human IFN- $\alpha$ C ( $\alpha$ 10)	15.6%
11160-1	Human IFN- $\alpha$ J1 ( $\alpha$ 7)	14.2%
11180-1	Human IFN- $\alpha$ 4b ( $\alpha$ 4)	10.7%
11135-1	Human IFN- $\alpha$ G ( $\alpha$ 5)	3.7%
11145-1	Human IFN- $\alpha$ H2 ( $\alpha$ 14)	0.3%
11130-1	Human IFN- $\alpha$ F ( $\alpha$ 21)	0.1%
11190-1	Human IFN- $\alpha$ WA ( $\alpha$ 16)	0.1%
11150-1	Human IFN- $\alpha$ I ( $\alpha$ 17)	0.1%
11115-1	Human IFN- $\alpha$ B2 ( $\alpha$ 8)	0.0%
14110-1	Rhesus Monkey IFN- $\alpha$	10.6%
16100-1	Cynomolgus Monkey IFN- $\alpha$ A Ile16	6.0%

2.3 ng/ml of Cynomolgus IFN- $\beta$  was measured in tissue culture supernatant of a mammalian cell line expressing Cynomolgus IFN- $\beta$ . No other antigen was detected in the supernatant.

iii. Mouse IFN- $\alpha$  A, Mouse IFN- $\beta$ , Mouse IFN- $\gamma$ , and Mouse IFN- $\lambda$ 3

Independent curves, starting at 10  $\mu$ g/ml, of recombinant analytes listed on the next page were prepared in Sample Diluent. A separate standard curve was prepared using the Human IFN Multiplex Antigen Standard supplied in the product. The % recovery of those points with pixel intensities within the range of pixel intensities of corresponding human analytes in the curve prepared using the Human IFN Multiplex

Antigen Standard were averaged to estimate the % cross reactivity for each analyte.

<b>Catalog No.</b>	<b>Analyte</b>	<b>% Cross-reactivity</b>
12100-1	Murine IFN- $\alpha$ A	0.004%
12400-1	Murine IFN- $\beta$	0.0%
12500-1	Murine IFN- $\gamma$	0.0%
12820-1	Murine IFN- $\lambda$ 3	0.0%

### 3. Additional Studies

Serum samples from 27 Rheumatoid Arthritis (RA) patients' were tested. Only 1 sample gave false positive. The spike recovery was acceptable.

20 human plasma samples with constituents known to interfere in immunoassays were tested. No sample gave false positive.

Please note that detection of analytes in serum and plasma from patients on certain therapeutics can be affected due to the presence of antibodies against analytes from the multiplex in such samples.



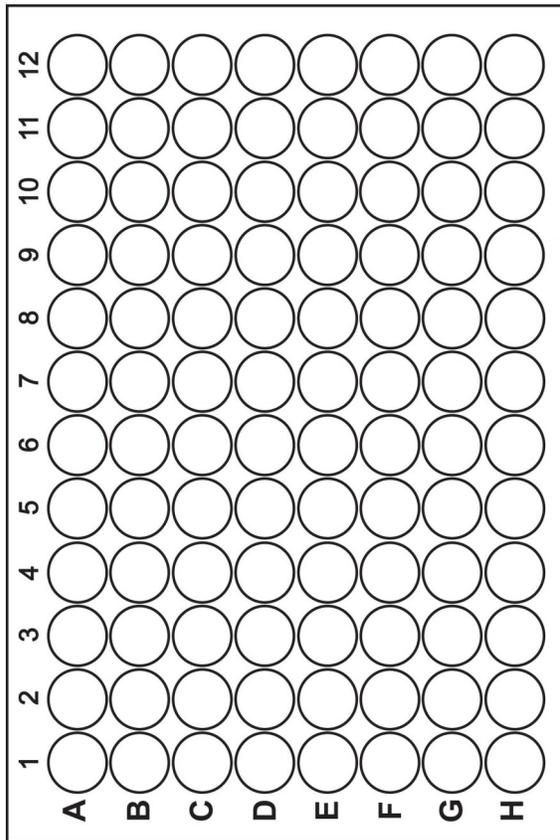
## G. REFERENCES

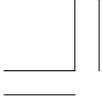
1. Evolution of the class 2 cytokines and receptors, and discovery of new friends and relatives; Pestka, S., *et al*; *Pharmacology & Therapeutics*; 2005 June; 106(3); 299-346.
2. Interferons, Interferon-like cytokines, and their receptors, Pestka, S., *et al*; *Immunological Reviews*; 2004; 202; 8-32.
3. Interferon Alpha in malignant and viral diseases: A review; Dorr RT; *Drugs*; 1993; 45(2).
4. Interferon-treatment for relapsing multiple sclerosis; Zivadinov, R., *et al*; *Expert Opinion in Biological Therapeutics*; 2008; 8(9); 1435-37.
5. Interferon- $\gamma$ : an overview of signals, mechanism, and functions; Hume, DA, *et al*; *Journal of Leukocyte Biology*; 2004; 75; 163-189.
6. IFN- $\lambda$ : Novel anti-viral cytokines; Paludan, SR, *et al*; *Journal of Interferon & Cytokine Research*; 2006 May; 26 (6);373-79.
7. IL-28, IL-29 and their class II cytokine receptor IL-28R; Sheppard, P., *et al*; *Nature Immunology*; 2002 Dec; 4; 63-68.
8. Molecular characterization and Antiviral activities of Type III interferons; Blecha, F., *et al*; *Journal of Interferon & Cytokine Research*; 2010 Nov; 30(11); 801-807.

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9. IFN-λs mediate antiviral protection through a distinct class II cytokine receptor complex; Donnelly, RP, *et al*; *Nature Immunology*; 2002 Dec; 4; 69-77.
  10. Human IFN-lambda 1 modulates the Th1/Th2 response; Gallagher, G., *et al*; *Genes & Immunity*; 2007 March; 8; 254-261.
  11. Lambda Interferon inhibits Hepatitis B and Hepatitis C Virus Replication; Chisari, FV, *et al*; *Journal of Virology*; 2005 March; 79(6); 3851-3854.
  12. Interferon Lambda as a potential new therapeutic for hepatitis C; Williams, DE, *et al*; *Annals of the New York Academy of Sciences*; 2009 Dec; 1182; 80-87.

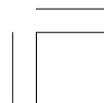
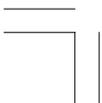
**PLATE LAYOUT**

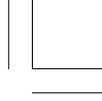
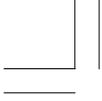
Use this plate layout as a record of standards and samples assayed.





**NOTES**





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