Erenna®

# IL-21 Immunoassay Kit

**Microparticle Assay**

Catalog #03-0014-07

Immunoassay kit for the quantitative determination of **IL-21** in EDTA plasma and serum

**FOR RESEARCH USE ONLY**

**NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC PROCEDURES**

**Manufactured & Distributed by:**

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05-0206-05

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# INTRODUCTION

The Erenna® IL-21 Immunoassay uses a quantitative fluorescent sandwich immunoassay technique to measure IL-21 in human K2 EDTA plasma samples. A capture antibody specific for IL-21 has been pre-coated onto paramagnetic microparticles (MP). The user pipettes MP, standards, and samples into uncoated microplate wells. During incubation, the IL-21 present in the sample binds to the capture antibody on the coated MP. Unbound molecules are washed away during the subsequent buffer exchange and wash steps. Fluor-labeled detection antibody is added to each well and incubated. This detection antibody recognizes and binds to IL-21 that has been captured onto the MP. During the following wash step the MPs are transferred to a clean plate. Elution buffer is then added and incubated. The elution buffer dissociates the bound protein sandwich from the MP surface, releasing the labeled antibodies. These antibodies are separated during transfer to a final microplate. The plate is loaded into the Erenna System where the labeled molecules are detected and counted. The number of fluor-labeled detection antibodies counted is directly proportional to the amount of IL-21 present in the sample when captured. The amount of IL-21 in unknown samples is interpolated from a standard curve.

# MATERIALS

The Erenna® IL-21 Immunoassay kit includes all reagents listed in Table 1: Reagent Data. Additional reagents and supplies may be required to run this immunoassay, as listed in APPENDIX C: Additional Supplies Required.

## Reagents Provided

| Item # | Description | Shipping Conditions | Storage Conditions | Component Part No. |
|---|---|---|---|---|
| 1 | **Human IL-21 Coated Beads** | With cold pack | 2-8$^{o}$C | 02-0220-04 |
| 2 | **Standard Diluent** | With cold pack | 2-8$^{o}$C | 02-0225-02 |
| 3 | **Assay Buffer** | With cold pack | 2-8$^{o}$C | 02-0231-02 |
| 4 | **Human IL-21 Standard** | On dry ice | ≤ -20$^{o}$C | 02-0224-00 |
| 5 | **Human IL-21 Labeled Detection Antibody** | With cold pack | 2-8$^{o}$C | 02-0222-03 |
| 6 | **Erenna® IL-21 Immunoassay Kit Instructions** | N/A | Ambient | 05-0206-05 |
| 7 | **10X Wash Buffer** | With cold pack | 2-8$^{o}$C | 02-0001-03 |
| 8 | **Elution Buffer B** | With cold pack | 2-8$^{o}$C | 02-0211-02 |
| 9 | **Buffer C** | With cold pack | 2-8$^{o}$C | 02-0230-01 |

**Table 1: Reagent Data**

## MATERIALS (continued)

## Storage Instructions

The **Erenna® IL-21** Immunoassay Reagent Kit should be stored at 2–8°C. The Standard analyte should be stored at ≤ -20°C. Proper kit performance can only be guaranteed if the materials are stored properly.

## General Supplies Required (manufacturer not specified)

- De-ionized or distilled water
- Multichannel pipette capable of transferring or adding 4 µL, 20 µL, 100 µL and 250 µL
- Micro-centrifuge tubes
- Micro-centrifuge
- Container capable of holding 300 mL
- 500 mL graduated cylinder
- Rotisserie rotator for bead resuspension

## TECHNICAL HINTS DUE TO HIGH SENSITIVITY

- Wipe down bench and pipettes with 70% isopropanol before use.
- Quickly spin concentrated standard before opening vials.
- Use sterile filter pipette tips and reagent trays to avoid contamination.
- Use filter tips while transferring concentrated standard.
- Use a 12-channel reservoir for preparing standards.
- Pre-wet tips (aspirate and dispense within well) twice before each transfer.

## ADDITIONAL SAMPLE INFORMATION

- The Erenna® IL-21 Immunoassay has been validated using EDTA plasma and serum.
- Ensure sample is clear of precipitants and other visible particulate matter before testing with the Erenna® IL-21 Immunoassay.

## PRECAUTIONS

- Use caution when handling biological samples; wear protective clothing and gloves.
- Components of this reagent kit contain approximately 0.1% sodium azide as a preservative. Sodium azide is a toxic and dangerous compound when combined with acids or metals. Solutions containing sodium azide should be disposed of properly.

# ASSAY PREPARATION

## Reagent Preparation

1. Warm the following reagents to room temperature prior to use: **Standard Diluent**, **Assay Buffer**, **Elution Buffer B**, **Buffer C** and **10X Wash Buffer**.
2. Place the following reagents at 4°C until ready to use: **IL-21 Detection Antibody**.
3. Prepare 1X Wash Buffer (from 10X Wash Buffer) as follows:
    a. Pour the contents of the 30 mL bottle of 10X Wash Buffer into a container capable of holding at least 300 mL
    b. Add 270 mL of deionized water
    c. Mix thoroughly by gentle inversion or with a clean, sterile stir bar
4. Mix **IL-21 Coated Beads** (coated microparticles) on a rotisserie spin rotator, or manually by repeat inversion, for 10-20 minutes until all MPs are completely resuspended.

## Sample Preparation

1. Prepare samples by one of the following methods:
    a. If using a filter plate with prefilter (Pall PN: 5041): Stack the Pall 5041 filter plate on top of a 96-well receptacle plate. Place 250 µL of sample into a filter plate well and spin for ≥ 10 minutes at 1,100 x *g*.
    b. If using a microcentrifuge: Centrifuge K2 EDTA plasma samples at >13,000 x *g* for 10 minutes immediately prior to use. Carefully pipette the supernatant into a clean microcentrifuge tube, avoiding particulates and slowly aspirating below the lipid layer.
2. Dilute the clarified samples 1:2 in the sample preparation plate using the **Standard Diluent** (e.g., transfer 175 µL of clarified sample to the sample preparation plate and add 175 µL **Standard Diluent**). Mix thoroughly before transferring to assay plate.

**Note:** During data analysis, the interpolated value of the diluted sample needs to be multiplied by 2 in order to calculate the correct concentration of the analyte in the sample.

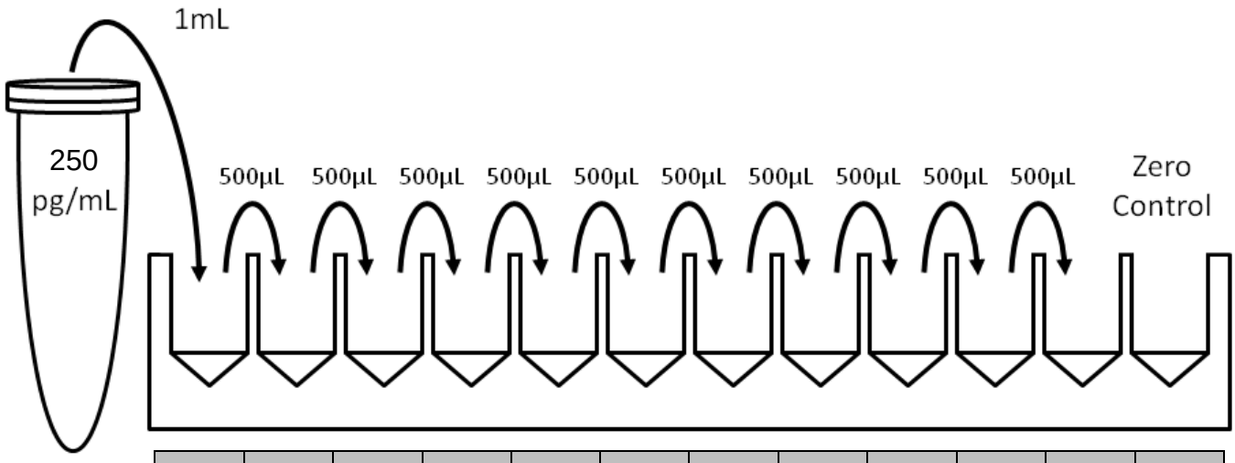
## Initial Standard Stock Preparation

1. Quick spin and pipette mix the **IL-21 Standard Analyte** vial in a mini-centrifuge prior to opening. Use care when opening this concentrated standard vial to prevent loss of materials and contamination of specimens or plates with aerosols.
2. Refer to the standard value assignment for the starting concentration of the **IL-21 Standard Analyte** in the vial.
3. To make your **Analyte Working Stock**, perform the necessary serial dilutions to achieve the final working concentration of 250 pg/mL in a 1 mL final volume. Ensure that all pipetting steps transfer ≥10 µL of liquid to achieve the best precision.

# IL-21 ASSAY PROCEDURE

## Standard Curve

Prepare the standard curve in a 12-channel reservoir dilution plate. Perform 1:3 serial dilutions of the **Analyte Working Stock** for dilutions 2 and 3 then 1:2 serial dilution for 4 to 11, to achieve a curve from 250 pg/mL to 0.11 pg/mL. Run the standards in triplicate.




| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 250 | 83.33 | 27.78 | 13.89 | 6.94 | 3.47 | 1.74 | 0.87 | 0.43 | 0.22 | 0.11 | 0 |

1. Add 1000 µL **Standard Diluent** to wells 2 and 3 of a 12-channel reservoir dilution plate.
2. Add 500 µL **Standard Diluent** to wells 4 through 12.
3. Add 1000 µL of the 250 pg/mL **Analyte Working Stock** from standard preparation into well 1.
4. Transfer 500 µL from well 1 into well 2, mixing thoroughly. Continue serial dilutions from well 2 stopping at well 11. **Use a fresh tip with each transfer**.

## Target Capture

1. Pipette 100 µL per well of Standards or diluted Samples to **Plate 1** (96-well PolyPropylene).
2. Mix microparticles (MPs) by gentle inversion until all MPs are completely resuspended.
3. Immediately before adding to the assay plate, add the vial of **IL-21 Coated Beads** to 10.5 mL of the supplied **Assay Buffer**. Mix by gentle inversion. Ensure that all beads have been transferred.
4. Pipette 100 µL per well of the resuspended MPs into **Plate 1**.
5. Cover **Plate 1** with an Axyseal plate cover.
6. Incubate for 2 hours at 25°C on Boekel Scientific, The Jitterbug™ setting 5.
7. Approximately 10 minutes prior to the end of Target Capture incubation, dilute concentrated **IL-21 Detection Antibody** by adding 10 µL of the reagent to 90 µL of **Assay Buffer**. Then add 30 µL of this intermediate dilution to 2970 µL of **Assay Buffer**. Filter the final diluted detection antibody using the syringe with a 0.2 µm filter into a clean tube.

8. When incubation is complete, carefully remove temporary plate cover to avoid splashing.

## Post-Capture Wash

1. If using a ***Bravo™ Liquid Handling Platform*** with the Singulex protocols installed, run protocol: ***80-0003-00_Bravo_protocol_ postCapture_1plate_v1a.pro***
2. If not, refer to **APPENDIX C: Manual Wash Protocols** and follow the **Manual Post-Capture Wash**.

## Detection

1. Immediately remove **Plate 1** from the magnet and add 20 µL per well of **IL-21 Detection Reagent**.
2. Cover **Plate 1** with an Axyseal plate cover.
3. Incubate for 1 hour at 25°C on Jitterbug setting #5.
4. Carefully remove Axyseal to avoid splashing.

## Post-Detection Wash Protocol

1. If using a ***Bravo™ Liquid Handling Platform*** with the Singulex protocols installed, run protocol: ***80-0015-01-Bravo_protocol_postDetection_1plate_v1c.pro***
2. If not, refer to **APPENDIX C: Manual Wash Protocols** and follow the **Manual Post-Detection Wash**.

## Elution

1. Immediately remove **Plate 2** from the magnet.
2. Add 20 µL **Elution Buffer B** per well.
3. Cover **Plate 2** with an AxySeal.
4. Incubate plate for ≥ 5 minutes at 25°C on Jitterbug setting 5.
5. Add 4 µL per well of **Buffer C** to assay **Plate 3** (384-well plate).
6. Remove AxySeal from **Plate 2**.
7. *If using a* ***Bravo™ Liquid Handling Platform*** with the Singulex protocols installed, run protocol: ***80-0005-00_Bravo_protocol_ elutionTransfer_1plate_v1a.pro***
8. If not, transfer plate manually.
9. Stack a 384-well filter plate on top of **Plate 3** containing 4 µL **Buffer C**
10. Transfer eluted product to the stacked plates.
11. Cover the top of stacked plate with Universal Plate Cover.
12. Spin plates for 1 minute at RT, approximately 1,100 x *g*.
13. Remove filter plate and discard.
14. Cover **Plate 3** with Heat Sealing Foil, according to manufacturer instructions for the heat sealer.

## Run on Erenna Immunoassay System

1. Load completed assay **Plate 3** onto the Erenna Immunoassay System.

## APPENDIX A: ERENNA® Quick Assay Guide

1. Prepare all reagents, standard curve, and samples as instructed.
2. Add 100 µL of Standard/diluted Samples and 100 µL of diluted **Capture Reagent** to **Plate 1**.
3. Cover and incubate for 2 hours at 25°C on Jitterbug (setting 5).

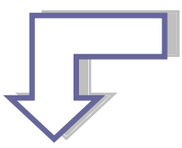
2 Hours
25°C

4. Perform Post-Capture Wash (**Plate 1**).
5. Remove from magnet and add 20 µL of diluted **Detection Reagent** per well.
6. Cover and incubate for 1 hour at 25°C on Jitterbug (setting 5).

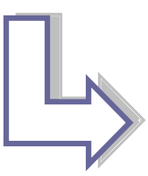
1 Hour
25°C

1. Perform Post-Detection Wash (**Plate 2**).
2. Remove from magnet and add 20 µL of **Elution Buffer B** to each well.
3. Cover and incubate at 25°C for ≥5 minutes on Jitterbug (setting 5).

5 Minutes
25°C



1. Add 4 µL **Buffer C** per well to **Plate 3**.
2. Stack a 384-well filter plate over **Plate 3**.
3. Transfer contents of 96-well **Plate 2** to **Plate 3** stack.
4. Cover filter, centrifuge stack for 1 minute at 1,100 x *g*.
5. Remove top filter plate and discard.
6. Cover assay **Plate 3** with pierceable plate seal cover.



**LOAD ON ERENNA® SYSTEM**

# APPENDIX B: Additional Supplies Required (not provided)

| Description | Mfr Supplier | Component Part Numbers | Product Uses | Packaging Detail |
|---|---|---|---|---|
| Erenna® 10X Systems Buffer | Singulex | 02-0111-00, 02-0111-01 | Systems (Analysis) Buffer, fluid used to run Erenna System | 1L (10L mixed) 2L (20L mixed) |
| Reservoirs for 12-Channel Pipetters | VWR | 80092-466 | Standard Curve | 10/pkg |
| 96-Well V-Bottom PolyPropylene Plate, 480 µL | Axygen | P-96-450V-C or P-96-450V-C-S | Assay plates, Receptacle plates | 10 plates/unit 5 units/case |
| 8-Well Low Profile Reservoir | VWR | 12000-732 | Transfer of Reagents | Variable |
| 384-Well Round Bottom PolyPropylene Plate, 120 µL | Nunc | 264573 | Receiver/analysis plate | 20/pk or 120/cs |
| Syringe (5 ml) | VWR | 66064-772 (or equivalent) | To filter diluted detection antibody | 100 units/pk |
| 0.2 µm Syringe Filter | Pall | 4187 | To filter diluted detection antibody | 50/pk |
| AcroPrep™ 384-Filter Plates, 100 µL, for sample preparation and detection | Pall | 5070 | Remove MPs from assay | 10/pkg |
| AcroPrep™ 96-well Filter Plate (Supor Membrane) | Pall | 5041 | Alternate sample preparation | 10/pkg |
| Universal Plate Cover | Nunc | 253623 | Cover the plate | 25 units/pk |
| AxySeal—PCRSP Plate sealing film series | Axygen | PCR-SP | Sealing plates during incubation/ mix/store | 100 films/ case |
| Dynal MPC® - 96S | Dynal™ | 120.27 | Rare Earth Magnet to capture MP during wash | 1 plate |
| Microplate Wash Station | Velocity11 Bravo | - - - | Wash MP following capture on magnet | - - - |
| Centrifuge w/ Plate Rotor | - - - | - - - | Remove MP via filter plate ~1,100 xg | 1 |
| Centrifuge Adapter Collar | Pall | 5225 | Creates fit b/n 384-well filter plate, 384-well assay plate | 2/pkg |
| Vacuum Pump | Welch | 2511B-01 | Degassing systems buffer | 1 |
| Microplate Incubator / Shaker | Boekel Scientific | 130000 The Jitterbug™ | Incubating plate | 1 |
| Heat Sealing Plate Foil | Singulex | 01-0216-00 or equivalent | Sealing plate for analysis on Erenna | - - - |
| Heat Sealer with adjustable temperature and time | FluidX | XTS-384 or equivalent | Sealing plate for analysis on Erenna | 1 |
| Universal Adapter for SBS format plates | FluidX | 42-1001 or equivalent | Required for proper sealing on XTS-384 | 1 |

# APPENDIX C: Manual Wash Protocols

## Manual Post-Capture Wash:

1. Place **Plate 1** onto magnet (Dynal MPC® - 96S)
2. Wait 2 minutes for MP to settle (ensure all MP are amassed as a pellet by magnet)
3. Aspirate the supernatant (MP remain visible)
4. Add 200 µL of Wash Buffer
5. Wait ≥ 1 minute, to be sure that the MPs remain amassed
6. Aspirate buffer

## Manual Post-Detection Wash:

1. Place **Plate 1** onto magnet
2. Wait 2 minutes
3. Aspirate the supernatant and discard into waste, change tips
4. Add 200 µL of Wash Buffer to each well
5. Wait ≥ 1 minute, to be sure that the MPs remain amassed, do not suspend or remove MP from the magnet during this time
6. Aspirate buffer from each well, discard into waste and change tips
7. Repeat steps 5 – 7 two more times for a total of three washes
8. Add 200 µL of Wash Buffer to each well of **Plate 1**
9. Mix gently by pipetting up and down to re-suspend MP pellet, ensuring that the pellet is fully resuspended
10. Transfer contents of each well to 96-well PolyPropylene Plate (**Plate 2**) on magnet
11. Add 200uL of Wash Buffer to each well of **Plate 1**
12. Wait 2 minutes for MP to amass/settle in **Plate 2**
13. Aspirate Wash Buffer from Plate 2, discard into waste and change tips
14. Mix **Plate 1** gently by pipetting to re-suspend any remaining MP
15. Transfer contents of each well to **Plate 2**
16. Add 200uL Wash Buffer to **Plate 1** and return to magnet
17. Remove **Plate 2** from the magnet mix gently by pipetting to re-suspend MPs.
18. Place **Plate 2** on magnet
19. Wait 2 minutes for MP to amass/settle
20. Aspirate Wash Buffer and discard into waste
21. Repeat steps 9 – 20 two more times for a total of three washes
22. Inspect **Plate 1** for any remaining MPs
    a. Yes, MPs are present in **Plate 1**:
        - Remove **Plate 1** from magnet. Gently mix by pipetting to re-suspend the MP pellet.
        - Transfer the contents of each well containing MPs to **Plate 2** on magnet.
        - Wait 2 minutes for MPs to amass/settle in **Plate 2.**
        - Aspirate Wash Buffer and discard into waste

    b. No, MPs are not present in **Plate 1.**
        - Discard Plate 1
23. Magnetized MP pellet should be visible in **Plate 2.**

## CONTACT INFORMATION

To reach Singulex, Inc. reagent technical support, call **(510) 995-9000**, or in the U.S. you may call us toll-free at (888) 603-3033.

You can also send us an e-mail at **techsupport@singulex.com**

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