# Quantikine® ELISA

## Mouse IL-27 p28/IL-30 Immunoassay

Catalog Number M2728

For the quantitative determination of mouse Interleukin 27 p28 subunit (IL-27 p28) concentrations in cell culture supernates, serum, and plasma.

**Note: The standard reconstitution method has changed. Read this package insert in its entirety before using this product.**

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

# TABLE OF CONTENTS




**MANUFACTURED AND DISTRIBUTED BY:**

**USA & Canada | R&D Systems, Inc.**
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

**DISTRIBUTED BY:**

**UK & Europe | R&D Systems Europe, Ltd.**
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

**China | R&D Systems China Co., Ltd.**
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

## INTRODUCTION

Interleukin 27 (IL-27) is a non-covalently linked heterodimeric cytokine that is structurally related to IL-12 (1). It is composed of an Epstein-Barr virus-induced molecule 3 (EBI3) subunit linked with the IL-27 p28 subunit (2-4). IL-27 p28, a 28 kDa glycoprotein belonging to the type I cytokine family, is homologous to IL-12 p35, IL-23 p19, and IL-6 (5). EBI3, a 34 kDa glycoprotein containing two fibronectin type III domains, belongs to the type I cytokine receptor family. It is homologous to the p40 subunit of IL-12 and IL-23 and to the extracellular domain of IL-6 Rα (6). In addition to IL-27 p28, EBI3 heterodimerizes with IL-12 p35 and can also exist as a homodimer (7). IL-27 is produced by activated antigen presenting cells including monocytes, endothelial cells, and dendritic cells (8). Its expression is induced in response to various inflammatory stimuli (7, 8).

IL-27 binds and signals through a heterodimeric receptor complex consisting of WSX-1 (TCCR) and gp130, both belonging to the cytokine receptor superfamily (9, 10). WSX-1 is specific for IL-27 and is expressed on resting/naive $CD4^+$ T cells, $CD8^+$ T cells, NK cells, dendritic cells, monocytes, mast cells, and B cells (5, 9, 10). In contrast, gp130 is ubiquitously expressed by a variety of immune and non-immune cells and functions as a subunit of the receptor complexes for at least seven other cytokines (5). IL-27 has both pro- and anti-inflammatory properties (11). In response to infection, IL-27 induces monocytes and mast cells to secrete pro-inflammatory cytokines. It induces naive $CD4^+$ T cells to proliferate and develop Th1 cell responses. IL-27 also promotes effector functions of NK cells and $CD8^+$ T cells. As an anti-inflammatory immunomodulator, IL-27 has been found to have the ability to inhibit Th1 or Th2 responses and restrict the strength and duration of adaptive immune responses.

The Quantikine® Mouse IL-27 p28/IL-30 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse IL-27 p28 in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant mouse IL-27 p28 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse IL-27 p28 showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse IL-27 p28.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-27 p28 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-27 p28 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for mouse IL-27 p28 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of IL-27 p28 bound in the initial step. The sample values are then read off the standard curve.

## LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

## TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- For best results, pipette reagents and samples into the center of each well.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

## PRECAUTIONS

Some components of this kit contain sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

## MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

| PART | PART # | DESCRIPTION | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---|---|---|---|
| **Mouse IL-27 p28 Microplate** | 892958 | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IL-27 p28. | Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.* |
| **Mouse IL-27 p28 Standard** | 892960 | 2 vials of recombinant mouse IL-27 p28 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.* | Use a new standard and control for each assay. Discard after use. |
| **Mouse IL-27 p28 Control** | 892961 | 2 vials of recombinant mouse IL-27 p28 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label. | |
| **Mouse IL-27 p28 Conjugate** | 892959 | 12 mL of a monoclonal antibody specific for mouse IL-27 p28 conjugated to horseradish peroxidase with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| **Assay Diluent RD1X** | 895121 | 11 mL of a buffered protein base with preservatives. *May contain a precipitate. Warm to room temperature and mix well before and during use.* | |
| **Calibrator Diluent RD5-3** | 895436 | 21 mL of a buffered protein base with preservatives. | |
| **Wash Buffer Concentrate** | 895003 | 21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.* | |
| **Color Reagent A** | 895000 | 12 mL of stabilized hydrogen peroxide. | |
| **Color Reagent B** | 895001 | 12 mL of stabilized chromogen (tetramethylbenzidine). | |
| **Stop Solution** | 895174 | 23 mL of diluted hydrochloric acid. | |
| **Plate Sealers** | N/A | 4 adhesive strips. | |

* Provided this is within the expiration date of the kit.

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- **Polypropylene** test tubes for dilution of standards.

## SAMPLE COLLECTION & STORAGE

**The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.**

**Cell Culture Supernates** - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Serum** - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 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 or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Note:** *Citrate plasma has not been validated for use in this assay.*
*Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.*

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

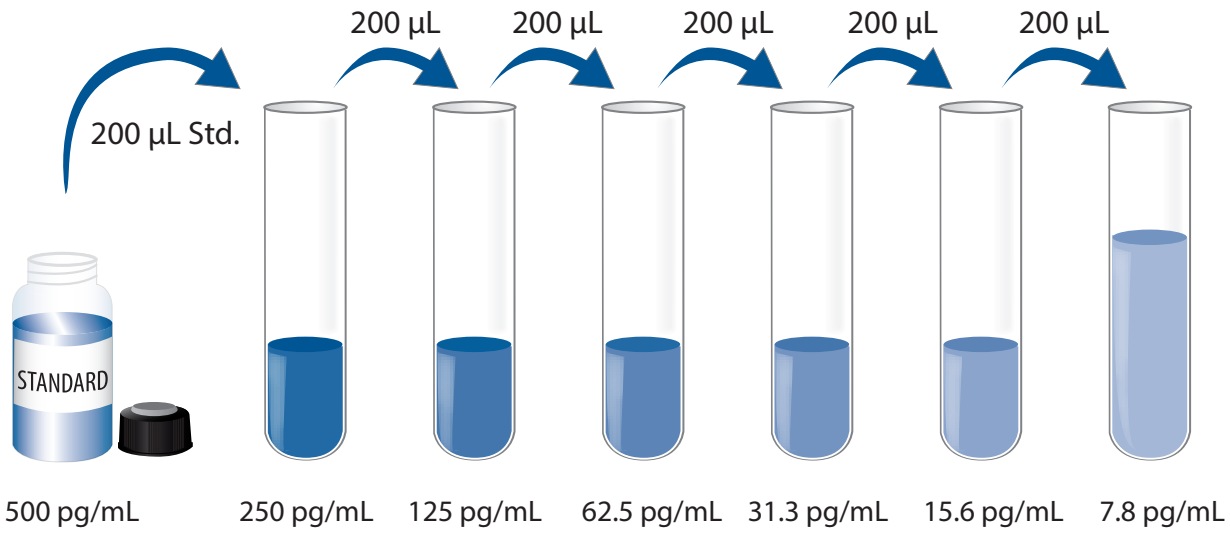
**Mouse IL-27 p28 Control** - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

**Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

**Mouse IL-27 p28 Standard** - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-27 p28 Standard with Calibrator Diluent RD5-3. Do not substitute other diluents. This reconstitution produces a stock solution of 500 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

**Use polypropylene tubes.** Pipette 200 µL of Calibrator Diluent RD5-3 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse IL-27 p28 Standard (500 pg/mL) serves as the high standard. Calibrator Diluent RD5-3 serves as the zero standard (0 pg/mL).



## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all standards, control, and samples be assayed in duplicate.**

1. Prepare all reagents, standard dilutions, control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1X to each well. *Assay Diluent RD1X may contain a precipitate. Mix well before and during use.*
4. Add 50 µL of standard, control, or sample per well. Tap the plate gently for one minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 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.
6. Add 100 µL of Mouse IL-27 p28 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

## CALCULATION OF RESULTS

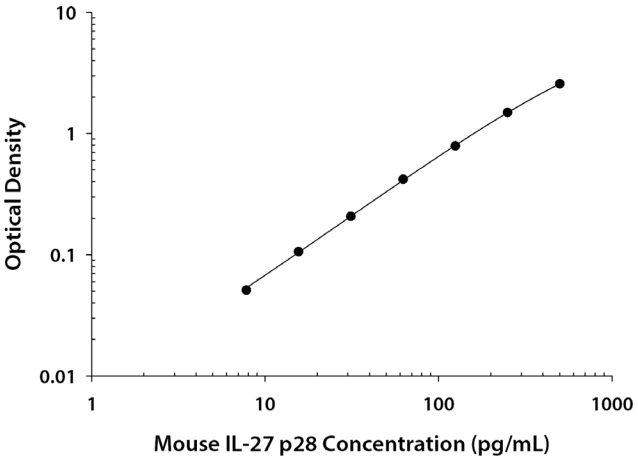
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse IL-27 p28 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## TYPICAL DATA

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




| (pg/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.051<br>0.058 | 0.055 | — |
| 7.8 | 0.099<br>0.111 | 0.105 | 0.050 |
| 15.6 | 0.156<br>0.164 | 0.160 | 0.105 |
| 31.3 | 0.258<br>0.264 | 0.261 | 0.206 |
| 62.5 | 0.463<br>0.484 | 0.474 | 0.419 |
| 125 | 0.842<br>0.847 | 0.845 | 0.790 |
| 250 | 1.501<br>1.589 | 1.545 | 1.490 |
| 500 | 2.603<br>2.648 | 2.626 | 2.571 |

## PRECISION

**Intra-assay Precision** (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

**Inter-assay Precision** (Precision between assays)
Three samples of known concentration were tested in separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of kit components.

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| **Sample** | 1 | 2 | 3 | 1 | 2 | 3 |
| **n** | 20 | 20 | 20 | 31 | 33 | 37 |
| **Mean (pg/mL)** | 20.0 | 49.2 | 228 | 24.1 | 56.7 | 238 |
| **Standard deviation** | 1.8 | 3.4 | 14.6 | 2.3 | 4.5 | 21.3 |
| **CV (%)** | 9.0 | 6.9 | 6.4 | 9.5 | 7.9 | 8.9 |

## RECOVERY

The recovery of mouse IL-27 p28 spiked to levels throughout the range of the assay in various matrices was evaluated.

| Sample Type | Average % Recovery | Range |
|---|---|---|
| Cell culture supernates (n=8) | 102 | 83-112% |
| Serum (n=8) | 95 | 85-102% |
| EDTA plasma (n=8) | 99 | 83-116% |
| Heparin plasma (n=8) | 95 | 87-101% |

## LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse IL-27 p28 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

| | | Cell culture supernates (n=11) | Serum (n=6) | EDTA plasma (n=6) | Heparin plasma (n=6) |
|---|---|---|---|---|---|
| 1:2 | Average % of Expected | 100 | 109 | 107 | 104 |
| | Range (%) | 92-117 | 105-112 | 101-115 | 101-112 |
| 1:4 | Average % of Expected | 96 | 108 | 105 | 105 |
| | Range (%) | 90-106 | 104-112 | 99-112 | 98-112 |
| 1:8 | Average % of Expected | 94 | 107 | 102 | 102 |
| | Range (%) | 87-110 | 99-112 | 96-109 | 92-111 |
| 1:16 | Average % of Expected | 103 | 109 | 100 | 109 |
| | Range (%) | 87-117 | 105-113 | 90-110 | 101-118 |

## SENSITIVITY

Eighteen assays were evaluated and the minimum detectable dose (MDD) of mouse IL-27 p28 ranged from 0.3-4.7 pg/mL. The mean MDD was 1.5 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

## CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant mouse IL-27 p28/IL-30 produced at R&D Systems®.

## SAMPLE VALUES

**Serum/Plasma** - Sixteen individual mouse serum, EDTA plasma, and heparin plasma samples were evaluated for detectable levels of mouse IL-27 p28 in this assay. All samples read below the low standard, 7.8 pg/mL.

**Cell Culture Supernates:**
J774 A.1 mouse reticulum cell sarcoma macrophage cells were cultured for three days in RPMI supplemented with 10% fetal bovine serum and stimulated with 100 ng/mL of recombinant mouse IFN-γ and 1.0 µg/mL of LPS. An aliquot of the cell culture supernate was removed, assayed for mouse IL-27 p28, and measured 302 pg/mL.

RAW 264.7 mouse monocyte/macrophage cells were cultured for four days in DMEM supplemented with 10% fetal bovine serum and stimulated with 1.0 µg/mL of LPS. An aliquot of the cell culture supernate was removed, assayed for mouse IL-27 p28, and measured 5959 pg/mL.

## SPECIFICITY

This assay recognizes natural and recombinant mouse IL-27 p28.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse IL-27 p28 control were assayed for interference. No significant cross-reactivity or interference was observed.

**Recombinant mouse:**
IL-6
IL-6 R
IL-11
IL-12
IL-12 p35
IL-12/IL-23 p40
IL-23
IL-23 p19
IL-23 R

**Recombinant rat:**
CNTF
IL-6
IL-12 p35
IL-12 p40
IL-23 p19

**Recombinant human:**
IL-12

Recombinant mouse IL-27 (EBI3/p28 fusion protein) cross-reacts approximately 7% in this assay.

Recombinant human IL-12/IL-23 p40 cross-reacts approximately 0.5% in this assay.

Recombinant human IL-27 (EBI3/p28 fusion protein) interferes at concentrations > 12,500 pg/mL in this assay.

## REFERENCES


1. Boulay, J-L. *et al.* (2003) Immunity **19**:159.
2. Trinchieri, G. *et al.* (2003) Immunity **19**:641.
3. Murakami, M. *et al.* (2004) Growth Factors **22**:75.
4. Cordoba-Rodriguez, R. and D.M. Frucht (2003) Expert Opin. Biol. Ther. **3**:715.
5. Pflanz, S. *et al.* (2002) Immunity **16**:779.
6. Nomura, H. *et al.* (1997) GenBank Accession #:O35228.
7. Holscher, C. (2004) Med. Microbiol. Immunol. (Berl).**193**:1.
8. Villarino, A.V. *et al.* (2004) J. Immunol. **173**:715.
9. Pflanz, S. *et al.* (2004) J. Immunol. **172**:2225.
10. Scheller, J. *et al.* (2005) Biochem. Biophys. Res. Commun. **326**:724.
11. Hunter, C.A. (2005) Nature Reviews Immunol. **5**:521.