Rat IL-12+p40 ELISA Kit

Catalog Number KRC0121 (96 tests), KRC0122 (2 × 96 tests)

Pub. No. MAN0014367 **Rev.** 4.0 (30)

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Rat IL-12+p40 ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of rat IL-12 and free p40 subunit in rat serum, EDTA plasma, buffered solution, or cell culture medium. The assay recognizes both natural and recombinant IL-12, and the free p40 subunit.

Interleukin-12 (IL-12) is a heterodimeric cytokine that is produced by phagocytic cells, antigen-presenting cells and B lymphocytes. The active form of IL-12 is a disulfide-linked, 70 kDa (p70) glycoprotein composed of 40 kDa (p40) and 35 kDa (p35) subunits.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KRC0121 (96 tests)
Rt IL-12 Standard, lyophilized.	2 vials
Standard Diluent Buffer; contains 8 mM sodium azide	25 mL
Antibody-Coated Wells, 96-well plate	1 plate
Rt IL-12 Biotin Conjugate; contains 8 mM sodium azide	11 mL
Streptavidin-Peroxidase (HRP) (100X); contains 3.3 mM thymol	0.125 mL
Incubation Buffer; contains 8 mM sodium azide	11 mL
Streptavidin-Peroxidase (HRP) Diluent; contains 3.3 mM thymol	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	4

Materials required but not supplied

- Distilled or deionized water
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Pre-dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

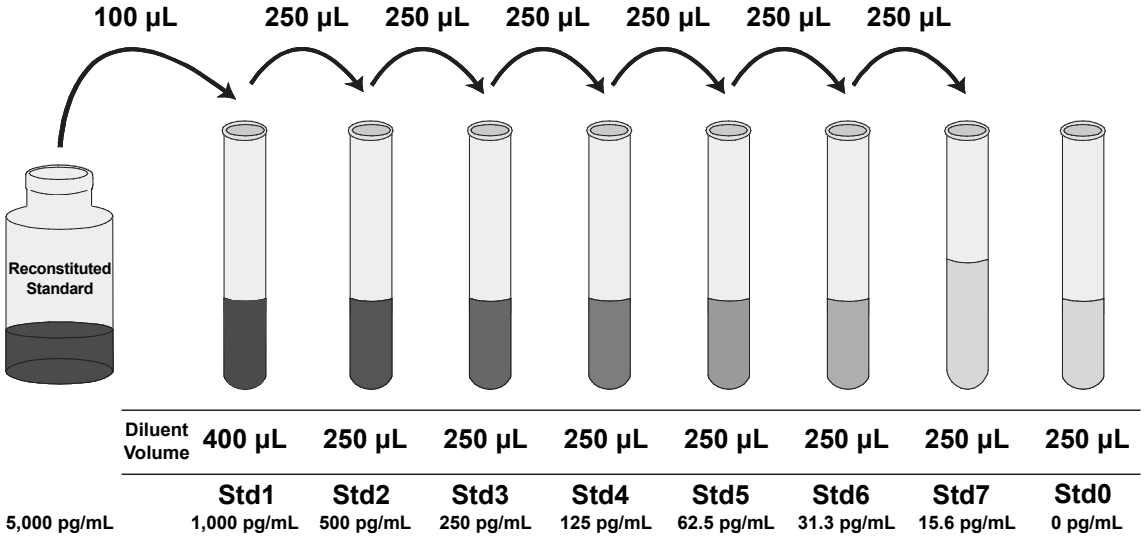
- Dilute samples with Standard Diluent Buffer (serum/plasma) or with the corresponding culture medium (cell culture supernatant).
- Dilute serum/plasma samples at least 1:5 in Standard Diluent Buffer.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

Note: The Rt IL-12 Standard was calibrated against a highly purified baculovirus-expressed recombinant protein from S*f*9 culture supernatant.

1. Reconstitute Rt IL-12 Standard to 5,000 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 5,000 pg/mL rat IL-12+p40. **Use the standard within 1 hour of reconstitution.**
2. Add 100 µL Reconstituted Standard to one tube containing 400 µL Standard Diluent Buffer and mix. Label as 1,000 pg/mL rat IL-12+p40.
3. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 500, 250, 125, 62.5, 31.3, 15.6, and 0 pg/mL rat IL-12+p40.
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. Discard any remaining reconstituted standard. Return the Standard Diluent Buffer to the refrigerator.



Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

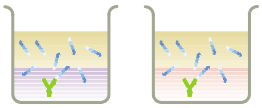
1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen

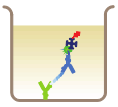


a. Add 100 µL of standards, samples, or controls to the appropriate wells.
b. Add 50 µL of **Incubation Buffer** to wells containing standards, serum/plasma, and controls, or 50 µL of **Standard Diluent Buffer** to the wells containing cell culture media. Leave the wells for chromogen blanks empty.
c. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
d. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Biotin Conjugate

a. Add 100 µL Rt IL-12 Biotin Conjugate solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 1 hour at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 Add Streptavidin-HRP



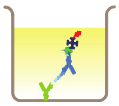
a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

4 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

5 Add Stop Solution



Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer (serum/plasma) or with the corresponding cell culture medium (cell culture supernatant) and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

The following data were obtained for the various standards over the range of 0 to 1,000 pg/mL rat IL-12.

Standard Rat IL-12 (pg/mL)	Optical Density (450 nm)
1,000	2.93
500	1.65
250	0.92
125	0.54
62.5	0.28
31.3	0.20
15.6	0.12
0	0.07

High-dose hook effect

A sample spiked with 50 ng/mL of rat IL-12 gave a response greater than that obtained for the highest standard data point.

Inter-assay precision

Samples were assayed 22 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	94	325	769
Standard Deviation	6.0	18.1	24.6
% Coefficient of Variation	6.4	5.6	3.2

Intra-assay precision

Samples of known rat IL-12 concentration were assayed in replicates of 22 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	89	344	814
Standard Deviation	4.0	11.3	38.2
% Coefficient of Variation	4.5	3.3	4.7

Expected values

Twelve pools of rat serum were evaluated in this assay. The values ranged from 770 to 2,200 pg/mL (mean 1,362 pg/mL).

Rat whole blood (WB) cells were cultured in RPMI supplemented with LPS and PHA, or PMA and ionomycin. Cell culture supernatant was collected at 24, 48, or 72 hours and assayed for released IL-12 using the Rat IL-12+p40 ELISA Kit.

Culture conditions for rat WB cells	Rat IL-12+p40 (pg/mL)		
	24 hr	48 hr	72 hr
None	59	38	46
LPS (25 µg/mL) + PHA (5 µg/mL)	211	363	309
PMA (100 ng/mL) + ionomycin (100 ng/mL)	222	212	231

Rat splenocytes were cultured at different cell densities in RPMI supplemented with 5% FCS, LPS (25 µg/mL), and PHA (5 µg/mL). Cell culture supernatant was collected at 24, 48, 72, or 96 hours and assayed for released IL-12 using the Rat IL-12+p40 ELISA Kit.

Splenocyte cell density	Rat IL-12+p40 (pg/mL)			
	24 hr	48 hr	72 hr	96 hr
0.25 x 10^6 cells/mL	314	450	472	456
0.8 x 10^6 cells/mL	676	ND	884	920
2.5 x 10^6 cells/mL	1,080	1,280	1,368	1,372

Recovery

The recovery of rat IL-12 added to rat serum, plasma, or cell culture medium containing fetal calf serum (FCS) was measured with the Rat IL-12+p40 ELISA Kit.

Sample	Average % recovery
Serum	95
EDTA plasma	96
Cell culture medium + 1% FCS	96
Cell culture medium + 10% FCS	105

Linearity of dilution

Rat serum and cell culture media samples were serially diluted in Standard Diluent Buffer or RPMI containing 1% fetal calf serum, respectively, over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded an average correlation coefficient of 0.99.

Dilution	Serum			Culture media		
	Measured (pg/mL)	Expected		Measured (pg/mL)	Expected	
		(pg/mL)	%		(pg/mL)	%
Neat	1,347	—	—	1,219	—	—
1/2	679	674	101	566	610	93
1/4	340	337	101	303	305	99
1/8	171	168	102	143	153	93
1/16	90	84	107	84	76	110

Sensitivity

The analytical sensitivity of this assay is <3 pg/mL rat IL-12 . This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

Specificity

Buffered solutions of a panel of substances at 100 ng/mL were assayed with the Rat IL-12+p40 ELISA Kit. The following substances were tested and found to have no cross-reactivity: **rat** IL-1β, IL-2, IL-4, IL-6, IL-10, IL-18, MIP-2, TNF-α, CINC-2β, VEGF, IFN-γ; **mouse** IFN-γ; IL-12, IL-15, IL-18, VEGF; **swine** IL-1β, IL-4, IL-6, IL-8, IL-15, IFN-γ, TNF-α and **human** IL-12.

Both *E. coli* and baculovirus derived rat IL-12 were detectable with this kit.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.