Quantikine® ELISA

Mouse IL-7 Immunoassay

Catalog Number M7000

For the quantitative determination of mouse Interleukin 7 (IL-7) concentrations in cell culture supernates, serum, and plasma.

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 7 (IL-7), previously known as pre-B-cell growth factor and lymphopoietin-1, is a type I short-chain cytokine belonging to the hematopoietin family (1-6). Mouse IL-7 is an approximately 25 kDa monomeric glycoprotein that is synthesized as a 154 amino acid (aa) residue precursor with a 25 aa signal sequence and a 129 aa mature segment. Mature mouse IL-7 has six cysteine residues that form three intrachain disulfide bonds, only one of which is required for bioactivity (7). Although multiple alternatively spliced isoforms of human IL-7 that differ in their receptor-binding affinities exist, alternatively spliced isoforms of mouse IL-7 have not been reported (8, 9). Mature mouse IL-7 shares 88% and 65% aa identity with rat IL-7 and human IL-7, respectively (8, 10). Cells known to express IL-7 include Class II MHC positive thymic cortical epithelial cells (11), follicular dendritic cells (12), intestinal epithelium (13), monocytes and keratinocytes (14, 15), endothelial cells (12, 16), vascular smooth muscle cells and fibroblasts (12), and bone marrow stromal cells (17).

The high-affinity receptor for IL-7 consists of two type I transmembrane glycoproteins, the ligand-binding IL-7 Rα and the non-binding common cytokine gamma chain (γc), both of which are required for signaling (1, 18, 19). The γc is a receptor subunit for multiple cytokines including IL-2, IL-4, IL-9, IL-15, and IL-21. IL-7 Rα is also shared and is utilized by thymic stromal-derived lymphopoietin (TSLP) in the receptor complex with TSLP receptor. Heparin/heparan sulfate proteoglycans have been shown to bind IL-7 and can regulate the bioavailability and bioactivity of IL-7 (20, 21).

IL-7 has a number of lymphocyte-associated activities. In the thymus, IL-7 induces the proliferation of triple negative immature thymocytes, participates in T cell receptor rearrangement, and suppresses CD4 expression in favor of CD8 on single positive T cells (1). In the periphery, IL-7 contributes to homeostatic proliferation and survival of naive $CD4^+$ and $CD8^+$ T cells (22-24), and promotes the formation and survival of resting memory $CD4^+$ T cells (25, 26) plus the proliferation of memory $CD8^+$ T cells (27). On B cells, IL-7 induces the differentiation of common lymphoid progenitors into $CD19^+$ B cell progenitors (28), and initiates the transition of pro-B cells into early pre-B cells (1). In the intestine, mucosal-derived IL-7 is crucial for both the development of intraepithelial γδ T cells and the organization of mucosal lymphoid tissue (29). On NK cells, IL-7 induces the proliferation and upregulation of the cytotoxicity of the $CD56^{bright}$ population of cells (30). This NK cell type is usually known for its cytokine production.

The Quantikine® Mouse IL-7 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse IL-7 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse IL-7 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse IL-7 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-7.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse IL-7 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-7 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-7 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-7 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.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between steps may improve assay precision.
- 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.

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-7 Microplate	892824	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse IL-7.	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-7 Standard	892826	Recombinant mouse IL-7 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Mouse IL-7 Control	892827	Recombinant mouse IL-7 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-7 Conjugate	892825	12 mL of a polyclonal antibody specific for mouse IL-7 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-21	895215	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-16	895302	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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards.

PRECAUTIONS

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.

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 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: *Heparin and citrate plasma have 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.

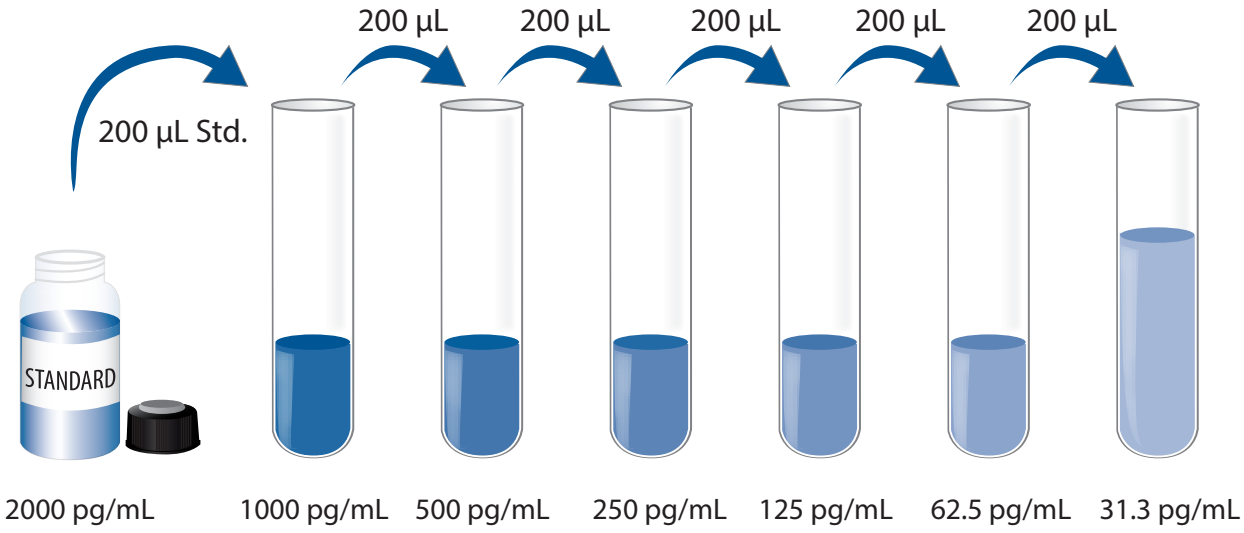
Mouse IL-7 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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-7 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-7 Standard with Calibrator Diluent RD5-16. Do not substitute other diluents. This reconstitution produces a stock solution of 2000 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 RD5-16 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-7 Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent RD5-16 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 RD1-21 to each well.
4. Add 50 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
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-7 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
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 **on the benchtop. 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

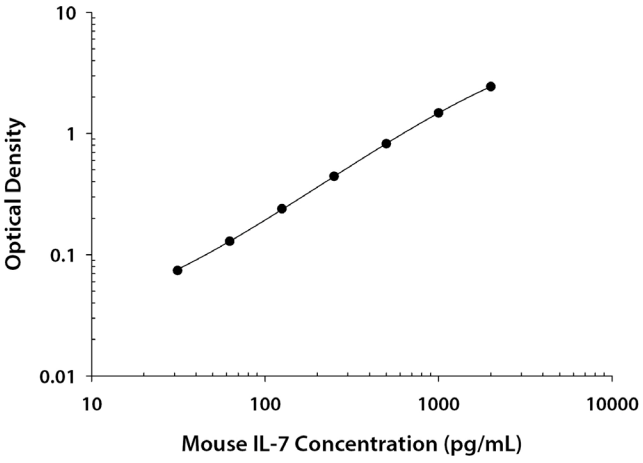
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-7 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	0.075 0.076	0.076	—
31.3	0.148 0.152	0.150	0.074
62.5	0.202 0.208	0.205	0.129
125	0.312 0.318	0.315	0.239
250	0.516 0.525	0.520	0.444
500	0.888 0.908	0.898	0.822
1000	1.553 1.559	1.556	1.480
2000	2.506 2.521	2.514	2.438

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 forty-one separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	41	41	41
Mean (pg/mL)	82	211	819	84	215	770
Standard deviation	3.5	5.9	29.6	6.7	16.6	67.1
CV (%)	4.3	2.8	3.6	8.0	7.7	8.7

RECOVERY

The recovery of mouse IL-7 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=6)	110	107-113%
Serum (n=4)	108	96-120%
EDTA plasma (n=4)	109	100-118%

LINEARITY

To assess linearity of the assay, samples were spiked with high concentrations of mouse IL-7 and diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)
1:2	Average % of Expected	92	96	99
	Range (%)	90-93	92-98	96-100
1:4	Average % of Expected	91	94	96
	Range (%)	84-96	93-94	96-97
1:8	Average % of Expected	90	95	96
	Range (%)	83-95	93-95	93-100
1:16	Average % of Expected	93	95	91
	Range (%)	80-104	92-97	86-94

SENSITIVITY

Fourteen assays were evaluated and the minimum detectable dose (MDD) of mouse IL-7 ranged from 3.5-8.3 pg/mL. The mean MDD was 6.3 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 *E. coli*-expressed recombinant mouse IL-7 produced at R&D Systems®.

SAMPLE VALUES

Serum - Twenty samples were evaluated for detectable levels of mouse IL-7 in this assay. Eighteen samples measured below the lowest standard, 31.3 pg/mL, and two samples measured 208 pg/mL and 56 pg/mL.

Plasma - Twenty-one samples were evaluated for detectable levels of mouse IL-7 in this assay. Eighteen samples measured below the lowest standard, 31.3 pg/mL, and three samples measured 69 pg/mL, 60 pg/mL, and 56 pg/mL.

Cell Culture Supernates - No detectable levels of mouse IL-7 were found in the following mouse cell culture supernates.

Cell Line
EL-4 mouse lymphoblast cells
IC-21 mouse macrophage cells
L-929 mouse fibroblast cells
WEHI-3 mouse myelomonocytic leukemia cells

SPECIFICITY

This assay recognizes natural and recombinant mouse IL-7.

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-7 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7 Rα
IL-9
IL-10
IL-11
IL-12
IL-12/IL-23 p40
IL-13
IL-15
IL-17
IL-18
IL-21
IL-22
TSLP

Recombinant human:

IL-7

REFERENCES

1. Fry, T.J. and C.L. Mackall (2002) Blood **99**:3892.
2. Appasamy, P.M. (1993) Cancer Invest. **11**:487.
3. Lupton, S.D. *et al.* (1990) J. Immunol. **144**:3592.
4. Namen, A.E. *et al.* (1988) Nature **333**:571.
5. VanderSpek, J.C. *et al.* (2002) Cytokine **17**:227.
6. Kroemer, R.T. *et al.* (1996) Protein Eng. **9**:493.
7. Cosenza, L. *et al.* (1997) J. Biol. Chem. **272**:32995.
8. Goodwin, R.G. *et al.* (1989) Proc. Natl. Acad. Sci. USA **86**:302.
9. Kroemer, R.T. *et al.* (1998) Protein Eng. **11**:31.
10. Widegren, B. *et al.* (2000) GenBank Accession #:P56478.
11. Oosterwegel, M.A. *et al.* (1997) Immunity **6**:351.
12. Kroncke, R. *et al.* (1996) Eur. J. Immunol. **26**:2541.
13. Watanabe, M. *et al.* (1995) J. Clin. Invest. **95**:2945.
14. Sieling, P.A. *et al.* (1995) J. Immunol. **154**:2775.
15. Heufler, C. *et al.* (1993) J. Exp. Med. **178**:1109.
16. Roye, O. *et al.* (1998) J. Immunol. **161**:4161.
17. Funk, P.E. *et al.* (1995) Blood **86**:2661.
18. Noguchi, M. *et al.* (1993) Science **262**:1877.
19. Goodwin, R.G. *et al.* (1990) Cell **60**:941.
20. Borghesi, L.A. *et al.* (1999) Blood **93**:140.
21. Clarke, D. *et al.* (1995) Cytokine **7**:325.
22. Schluns, K.S. *et al.* (2000) Nat. Immunol. **1**:426.
23. Boursalian, T.E. and K. Bottomly (1999) J. Immunol. **162**:3795.
24. Tan, J.T. *et al.* (2001) Proc. Natl. Acad. Sci. USA **98**:8732.
25. Kondrack, R.M. *et al.* (2003) J. Exp. Med. **198**:1797.
26. Li, J. *et al.* (2003) J. Exp. Med. **198**:1807.
27. Tan, J.T. *et al.* (2002) J. Exp. Med. **195**:1523.
28. Miller, J.P. *et al.* (2002) J. Exp. Med. **196**:705.
29. Laky, K. *et al.* (2000) J. Exp. Med. **191**:1569.
30. Dadmarz, R. *et al.* (1994) Lymphokine Cytokine Res. **13**:349.