

Interleukin-7 Active Protein | IL7 active protein

Mouse Interleukin-7 Recombinant

CATALOG #	HOST
AAA76391	E coli (Note: Host protein expression may be lot-specific and can be subject to change during production, including bulk orders.)

PRODUCT SYNONYMS

Interleukin-7; Mouse Interleukin-7 Recombinant; IL7 active protein

PRODUCT SPECIFICATIONS

Host	E coli (Note: Host protein expression may be lot-specific and can be subject to change during production, including bulk orders.)
Purity/Purification	>95%, as determined by SDS-PAGE and HPLC
Form/Format	Recombinant Interleukin-7 was lyophilized from a 0.2 um filtered solution in .5% glycine, .5% sucrose, .01% Tween80, mM Glutamic acid, pH.5.
Sequence	MFHVSFRYIF GIPPLILVLL PVTSSSECHIK DKEGKAYESV LMISIDELDK MTGTDSNCPN NEPNFFRKHV CDDTKEAFL NRAARKLKQF LKMNISEEFN VHLLTVSQGT QTLVNCTSKE EKNVKEQKKN DACFLKRLLR EIKTCWNKIL KGSI
Source	Optimized DNA sequence encoding Human Interleukin-7 mature chain was expressed in Escherichia Coli.
Endotoxin	Endotoxin content was assayed using a LAL gel clot method. Endotoxin level was found to be less than 0.1 ng/ug (1EU/ug).
Biological Activity	The ED(50) dose-dependent stimulation of the proliferation of murineE8 cells is <.1 ng/ml, corresponding to a specific activity of >4 x units/mg.

Method

T cell activation assay
Stromal cells: 24-well 1×10⁴ stromal cells from lines were seeded in complete RPMI and after overnight culture irradiated with 1000rad.

Initially, experiments with non-irradiated or irradiated pLN2 were performed with no difference in the outcome (data not shown).

Ex vivo stromal cells were isolated as described above and non-adherent cells washed away after overnight culture (density comparable with lines); they were non-proliferative and therefore not irradiated.

BMDC: They were activated with 0.5 ug/ml LPS for 6 h at 37 degree C.

2 h after LPS addition 1 uM SIINFEKL peptide was added.

1×10⁴ BM-DC were added per 24-well.

T cells: They were obtained ex vivo from spleen and pLN dissected from CO₂-killed WT B6 and OT-I transgenic mice and suspended by meshing.

Erythrocytes were removed using red blood cell lysis buffer (Tris-Ammonium chloride based).

CD8 cells were enriched by panning using antibodies to B220 (RA3-6B2), CD4 (H129.19.6), CD11b (M1/70) and CD11c (N418). OT-I cells were labeled with 2 uM CFSE.

Only 50% of WT B6 T cells were CFSE-labeled to identify the peak of undivided cells, with the 50% unlabeled T cells showing the background fluorescence of T cells.

Per 24-well 0.04×10⁶ CFSE+ OT-I cells together with 0.98×10⁶ unlabeled B6 WT cells and 0.98×10⁶ CFSE+ B6 WT cells were added.

The assay was performed in complete RPMI enriched with 1×MEM, 3 ng/ml murine IL-7 and 10 U/ml human IL-2. After 2-4 days, cells were harvested, counted and analyzed by flow cytometry.

Transwell assays used were 0.4 um transwell chambers (HTS 24-well).

Blocking experiments used 10 uM indomethacin, 1 uM 1400W (?=dihydrochloride), 10 uM 1-Methyl-L-tryptophan (1-MT), 200 uM (S)-(2-Boronoethyl)-L-cysteine, 10 ug/ml anti-PD-L1 (MIH5, eBioscience), 20 ug/ml anti-IL-10 (kind gift from,,) or 30 ug/ml anti-TGFbeta (clone 1D11.16.8).

To better compare the decrease of T cell proliferation between experiments the attenuating effect by the stromal cells was defined as percent inhibition in divided OT-I T cell numbers relative to the 'no stroma' control: {1-(number of proliferated cells)stroma/(number of proliferated cells)no stroma}*100}.

The 'no stroma' control was considered as 0% inhibition; absence of proliferating OT-I cells was considered as 100% inhibition.
Functional assays
Bone marrow was harvested from C57BL/6 mice and plated in a six-well dish at 1×10⁶ cells/ml in 2?ml of lymphocyte media (RPMI 1640 supplemented with 10% FCS, 5?mM L-Alanyl-L-Glutamine, 100?IU/ml penicillin-streptomycin and 2?mM beta-mercaptoethanol with 5?ng/ml of Daedalus IL-7 or commercial IL-7.

Media with IL-7 was replaced at Day 3 and the resulting cell populations were evaluated by fluorescence-activated cell sorting (FACS) at Day 5.

Cells were stained with fluorescein isothiocyanate (FITC) anti-mouse IgM and PE anti-mouse B220 (BD) in FACS staining buffer (PBS with 2.5% FCS) and analyzed on an LSRII flow cytometer (BD) and using FlowJo software.

Recombinant human LIF was tested for its ability to maintain pluripotency of murine ES cells.

Briefly, murine ES cells (AB1 ES cells kindly provided by Steve Jones, University of Massachusetts) were plated on irradiated feeder cells with either commercial human LIF or Daedalus recombinant human LIF at 10?ng/ml.

Cells were split every 2 days with fresh media and stained with APC anti-SSEA1 (BD) for analysis by flow cytometry.
Differentiation of CD34+ cells
Erythroid differentiation was carried out on MS-5 stromal cells as previously described 4 cells were plated in 2 mL medium'>serum-free expansion medium containing 100 ng/ml SCF, 5 ng/ml IL-3, 3 U/ml erythropoietin (EPO), and 1 mM hydrocortisone and cultured in 6-wells plates at 37 degree C and 5% CO₂ for 4 days.

The cells were then diluted into 6 ml of the same medium in a 25 cm² flask and cultured for an additional 4 days. Expanded cells were washed with basal medium and resuspended in 25 ml of basal medium with 3 U/ml EPO and plated onto a newly confluent 75 cm² flask of MS-5 feeder cells and cultured for 3 days.

For final maturation, erythroid cells were co-cultured on feeder cells for 10 days using basal medium without cytokines with one medium change after 5 days at which time 2×10⁶ cells were harvested for nucleic acid purification.

T-cell differentiation was carried on OP9 stromal cells as previously described 4 nucleofected cells were resuspended in alphaMEM with 20% FBS, 5 ng/ml IL-7, and 5 ng/ml Flt3L (both,,) onto sub-confluent OP9-DLL1 cells.

Fresh media with cytokines was replaced every 3 to 4 days.

B-cell differentiation was carried out on MS-5 stromal cells as reported 5 nucleofected CD34+ cells were resuspended in 15 ml of alphaMEM with 10% FBS, 10 ng/ml SCF, and 10 ng/ml G-CSF and seeded onto a confluent MS-5 layer in a 75 cm² flask.

Ten milliliters of fresh medium was added after 7 days and cells were maintained with twice weekly half-media changes.

Myeloid and granulocyte differentiations were performed on MS-5 stromal cells as described 5 nucleofected CD34+ cells were seeded in 1 ml IMDM with 10% FBS, 2 mM L-alanyl-glutamine, 50 ng/ml SCF, and 50 ng/ml IL-3 in six-well plates.

After 3 days in culture, cells were washed and seeded into 25 cm² flasks in basal medium plus 50 ng/ml SCF, 50 ng/ml IL-3, and 10 ng/ml G-CSF.

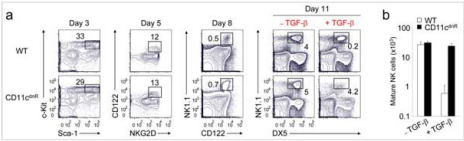
Cells were maintained at 2×10⁵ to 8×10⁵ cells per ml in complete medium with cytokines for 10 days until harvest.
In vitro Generation of NK cells

The generation of NK cells from stem cell precursors was conducted in vitro, based on protocol previously described. Briefly, CD11cdnR and wild-type mice were first treated (i.p.) with 5-FU (5-Fluorouracil) provided at 150 mg/kg body weight.

Four days later, mice were sacrificed and HSC-enriched bone marrow cells were cultured at 10,000 cells/well in

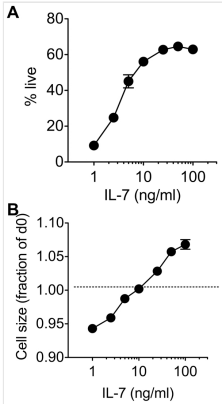
Molecular Function	Cytokine, Growth-factor
Reconstitution	A quick spin of the vial followed by reconstitution in distilled water to a concentration not less than 0.1 mg/mL. This solution can then be diluted into other buffers.
Preparation and Storage	The lyophilized protein is stable for at least years from date of receipt at -20 degree C. Upon reconstitution, this cytokine can be stored in working aliquots at 2 degree -8 degree C for one month, or at -20 degree C for six months, with a carrier protein without detectable loss of activity. Avoid repeated freeze/thaw cycles.

IMAGES



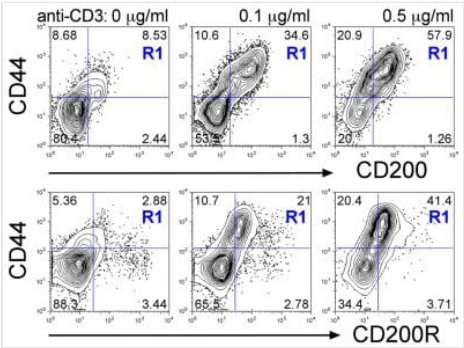
Application Data

TGF- β is a negative regulator of NK cell generation CD11cdnR and wild-type mice were injected (i.p.) Four days later, mice were sacrificed and HSC-enriched bone marrow cells were cultured in the presence of IL-7, SCF, and Flt3L.



Application Data

IL-7 mediated growth and survival is dose dependent in vitro. CD8 T cells were enriched from C57Bl6/J donors and cultured with a range of IL-7 concentrations.



Application Data

T cells co-express CD200 and CD200R following TCR activation. C. Naïve LN cells were stimulated with anti-CD3 (1 µg/ml) + anti-CD28 (2 µg/ml) either transiently on Ab-coated wells for the first 2d then removed to fresh media (blue lines) or chronically with Ab-coated aAPC present throughout the culture (red lines) in Th1 (top row), Th2 (middle row) or non-polarising (IL-7, bottom row) conditions.

ADDITIONAL INFORMATION

Product Categories/Family for IL7 active protein	Hematopoietins; IL-2 family; Recombinant Mouse Cytokines
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NCBI AND UNIPROT INFORMATION

NCBI GI #	6680433
NCBI GeneID	16196
NCBI Accession #	NP_032397.1
NCBI GenBank Nucleotide #	NM_008371.4
UniProt Accession #	P10168

Molecular Weight	17,727 Da
NCBI Official Full Name	interleukin-7
NCBI Official Synonym Full Names	interleukin 7
NCBI Official Symbol	IL7
NCBI Official Synonym Symbols	IL-7; hlb368; A630026I06Rik
NCBI Protein Information	interleukin-7
UniProt Protein Name	Interleukin-7
UniProt Gene Name	IL7
UniProt Synonym Gene Names	IL-7; IL-7
UniProt Entry Name	IL7_MOUSE

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.

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