Datasheet for ABIN2659081

anti-IL-6 Receptor antibody (PE-Cy7)

1 Validation

Go to Product page

Overview

Quantity:	100 tests
Target:	IL-6 Receptor (IL6R)
Reactivity:	Human
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This IL-6 Receptor antibody is conjugated to PE-Cy7
Application:	Flow Cytometry (FACS)

Product Details

Clone:	UV4
Isotype:	IgG1 kappa
Purification:	The antibody was purified by affinity chromatography and conjugated with PE/Cy7 under optimal conditions. The solution is free of unconjugated PE/Cy7 and unconjugated antibody.

Target Details

Target:	IL-6 Receptor (IL6R)
Alternative Name:	CD126 (IL6R Products)
Background:	CD126 is an 80 kD IL-6 receptor α chain also known as IL-6R. It is a member of the immunoglobulin superfamily that is expressed on plasma cells, T cells, activated B cells, monocytes, granulocytes, hepatocytes, epithelial cells, and fibroblasts. Functional IL-6 receptors are formed by the non-covalent association of CD126 and the IL-6 receptor β chain

Target Details

	(CD130 or gp130). CD126 binds IL-6 with low affinity but does not signal. The β chain (gp130, CD130) does not bind IL-6 by itself but associates with the α-chain/IL-6 complex to initiate signal transduction. IL-6 binding to the receptor complex results in the stimulation of B and T cells, and hematopoietic precursor proliferation and differentiation. A soluble form of CD126 has been found in human serum.
Pathways:	JAK-STAT Signaling, Autophagy, Growth Factor Binding, Cancer Immune Checkpoints

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Restrictions:	For Research Use only

Handling

Buffer:	Phosphate-buffered solution, pH 7.2, containing 0.09 % sodium azide and 0.2 % (w/v) BSA .
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Handling Advice:	Protect from prolonged exposure to light. Do not freeze.
Storage:	4 °C
Storage Comment:	The antibody solution should be stored undiluted between 2°C and 8°C.

Successfully validated (Flow Cytometry (FACS))

by Immunmodulatorische Abteilung, Universitätsklinikum Erlangen

Report Number: 101960

Date: Nov 06 2017

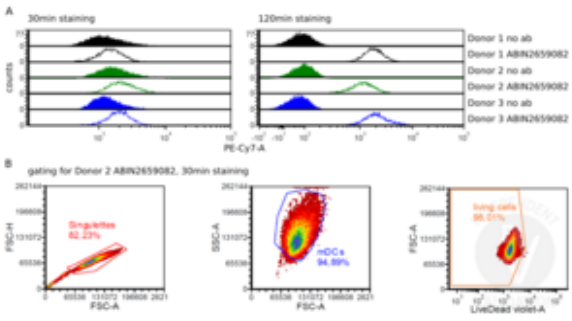
Target:	IL6R
Lot Number:	B236171
Method validated:	Flow Cytometry (FACS)
Positive Control:	Mature dendritic cells (mDCs) from three donors, incubated with ABIN2659082
Negative Control:	Mature dendritic cells (mDCs) from three donors, incubated without antibody
Notes:	Passed. ABIN2659082 specifically recognizes the IL-6 Receptor on mDCs.
Primary Antibody:	ABIN2659082
Protocol:	• Prepare peripheral blood mononuclear cells (PBMCs) from leukoreduction system chambers (LRSCs) (Pfeiffer et al. (2013); Weidinger et al. 2011) from three healthy donors (obtained following informed consent and approved by the institutional review board) by density centrifugation on a Lymphoprep gradient (Axis-shield AS, 1114547, lot 12IFS10). • For the generation of dendritic cells (DCs), prepare PBMCs from leukoreduction system chambers (LRSC) (Pfeiffer et al. (2013)). • After centrifugation, collect PBMCs forming a distinct band at the sample/Lymphoprep interface. • Wash PBMCs 3x with cold PBS supplemented with 1mM EDTA. • Wash PBMCs 1x with cold RPMI 1640 (Lonza, BE12-167F, lot 7MB088). • Resuspend PBMCs in warm DC Medium (RPMI 1640 supplemented with 1% AB-serum, 100U/ml penicillin, 100mg/ml streptomycin, 2mM L-glutamine, 10mM HEPES buffer (Lonza, BE17-737E)) and seeded into 175cm^2 tissue culture flasks at a density of 3.5x10^8 cells/flask. • Let monocytes adhere for 1h at 37°C and 5% CO_2. • Wash non-adherent fraction off with warm RPMI 1640. • Add 30ml DC medium supplemented with 800U/ml recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) and 250U/ml recombinant human interleukin-4 (IL-4) to each flask (day 1). • Incubate cells for 72h at 37°C and 5% CO_2. • On day 4 (or three days later), add 5ml of fresh culture medium containing GM-CSF (final concentration of 400 U/ml in 35ml) and IL-4 (final concentration of 250U/ml in 35ml) to the cells. • On day 5, mature the immature DCs by adding 10ng/ml recombinant human tumor necrosis factor alpha (TNF-α), 1µg/ml prostaglandin E2 (PGE2), 200U/ml recombinant human

interleukin-1β (IL-1β), 1000U/ml recombinant human interleukin-6 (IL-6), 40U/ml GM-CSF, and 250 U/ml IL-4 to the medium.

- Incubate cells for 48h at 37°C and 5% CO_2.
- On day 7, use the now mature DCs (mDCs) for FACS analysis.
- Analyze cell surface expression of IL-6 Receptor by FACS:
  - Harvest 2.5x10^5 mDCs by centrifugation.
  - Wash cells once with FACS buffer (PBS supplemented with 2% FCS).
  - Combine anti-human PE-Cy7 conjugated IL-6 Receptor antibody (antibodies-online, ABIN2659082, lot B236171) diluted 1:100 and LIVE/DEAD Violet dead cell stain kit (ThermoFisher Scientific, L34955, lot 1832693) diluted 1:300 in cold FACS buffer.
  - Resuspend mDCs in 100µl of the antibody-solution and incubate for 30min on ice in the dark.
  - Wash mDCs 2x with cold FACS buffer.
  - Fix mDCs using 2% paraformaldehyde in cold PBS.
  - Store mDCs at 4°C until analysis.
  - Run samples using a BD FACS Canto II and analyze events using FCS Express 5.

Experimental Notes:	Mature DCs were stained for FACS for the analysis of the expression of IL-6 Receptor/CD126 on the cell surface of the cells with ABIN2659082 for 30min or 120min respectively. The histogram shows a clearly shift between unstained control and the stained mock control by staining for 120min. The staining for 30min does also show a shift of the histogram peak, but not as clear as the staining for 120min.

Image for Validation report #101960



Validation image no. 1 for anti-Interleukin 6 Receptor (IL6R) antibody (PE-Cy7) (ABIN2659082)

A Flow cytometry analysis of mature DCs from three different donors after incubation for 30min or 120min with or without ABIN2659082. B Consecutive gating for single cells (left panel), mDCs (middle panel), and living cells (right panel), illustrated on donor 2 samples stained for 30min with ABIN2659082.