

KIR typing

Genotyping, mRNA expression profiling, and phenotyping of human NK cells

Natural killer (NK) cells represent an important lymphocyte population of the innate immune system. They have the capacity to lyse virus-infected cells and tumor cells without prior antigenic stimulation.

Negative regulation of NK cell function is mediated by inhibitory killer cell immunoglobulin-like receptors (KIRs) recognizing HLA I molecules on target cells. KIRs exist in various isoforms. Heterogeneity occurs at the genomic and transcriptional level in every human individual and also in different subsets of NK cells.

NK cells have been implicated in the suppression of graftversus-host disease, promotion of bone marrow engraftment, and mediation of graft-versus-leukemia effects in bone marrow transplantations, possibly as a result of donor-recipient HLA-KIR mismatching.

For KIR typing of NK cells at the DNA and mRNA level, as well as for phenotyping by flow cytometry, Miltenyi Biotec offers the KIR Typing Kit and a variety of antibodies, respectively.

The KIR Typing Kit—genotyping and mRNA expression profiling with a single kit

The KIR Typing Kit allows the detection of all known human KIR genes and alleles at the genomic DNA and mRNA level.

Accurate primer design for reliable results

The presence or absence of KIR genes is analyzed by PCR using sequence-specific primers (SSPs).

Carefully designed SSPs enable the detection of all 15 known human KIR genes plus two pseudogenes, and their alleles. Allele coverage is based on the IPD-KIR sequence database (http://www.ebi.ac.uk/ipd/kir).

Ready-to-use enzyme mix and reagents

The enzyme mix including Taq DNA Polymerase is lyophilized in each well of a 96-well PCR plate. Only resuspension buffer and the template of your choice need to be added.

Thanks to the integrated loading buffer, PCR products can be directly analyzed by gel electrophoresis.

Internal positive control

In addition to the KIR-specific primers, the wells contain positive control primers designed to indicate a successful PCR.

Lane	Gene name	Lane	Gene name	Lane	Gene name
1	2DL1	8	2DS1	15	3DL2
2	2DL2	9	2DS2	16	3DL3
3	2DL3	10	2DS3	17	3DS1
4	2DL4	11	2DS4del	18	2DP1
5	2DL5	12	2DS4ins	19	3DP1
6	2DL5A	13	2DS5		
7	2DL5B	14	3DL1		

 Table 1: KIR genes and variants whose presence or absence can be

 elucidated by the KIR Typing Kit. Lanes refer to figures 1 A and 1 B, overleaf.

MACS® Antibodies for KIR phenotyping

The KIR genotype of an NK cell population does not always reflect its phenotype. A broad panel of KIR-specific antibodies from Miltenyi Biotec enables the determination of KIR phenotypes of human NK cell populations by flow cytometry.

KIR genotyping



Figure 1 A: Gel electrophoresis of PCR products obtained from genomic DNA isolated from whole blood. Lanes 1–5, 7, 9–12, 14–16, and 18–19 are positive for KIR (+). Lanes 6, 8, 13, and 17 are negative (–). In addition, all lanes show positive control bands (400 bp). Lane 20 shows the genomic DNA control band at 260 bp, lane 21 the positive control, and lane 22 the negative control.

KIR phenotyping



Figure 2: Human PBMCs were stained with various anti-KIR antibodies and CD56-APC. Cells were analyzed by flow cytometry.

mRNA expression profiling of KIR genes



Figure 1 B: Gel electrophoresis of PCR products obtained from mRNA-derived cDNA isolated from 5 mL whole blood. Lanes 1–4, 9–12 and 14–15 are positive for KIR (+), whereas lanes 5–8, 13, and 16–19 are negative (–). In addition, all lanes include positive control bands (400 bp). Lane 20 shows no genomic DNA control band at 260 bp; thus, there is no genomic DNA contamination.

Product	Capacity	Order no.
KIR Typing Kit	8 tests 24 tests	130-092-551 130-092-584
MACS [®] Antibody	Conjugate	Order no.
CD158b (KIR2DL2/DL3), human	– PE – APC pure	130-092-618 130-092-617 130-092-615
CD158e (KIR3DL1), human	– FITC – PE – APC – Biotin pure	130-092-568 130-092-473 130-092-474 130-092-475 130-092-475 130-092-555
CD158a/h (KIR2DL1/DS1), human	– FITC – PE – APC – Biotin pure	130-092-811 130-092-684 130-092-685 130-092-683 130-092-683 130-092-682
CD158i (KIR2DS4), human	– PE – APC – Biotin pure	130-092-680 130-092-681 130-092-898 130-092-679
Anti-KIR2D, human	– PE – APC – Biotin pure	130-092-688 130-092-687 130-092-904 130-092-689

A complete list of products for the separation and analysis of NK cells is available at www.miltenyibiotec.com.

Miltenyi Biotec

Miltenyi Biotec GmbH Friedrich-Ebert-Straße 68 51429 Bergisch Gladbach Germany Phone +49 2204 8306-0 Fax +49 2204 85197 macs@miltenyibiotec.de

Miltenyi Biotec Inc. 12740 Earhart Avenue Auburn, CA 95602 USA Phone 800 FOR MACS, +1 530 888 8871 Fax +1 530 888 8925 macs@miltenyibiotec.com

Miltenyi Biotec Australia Pty. Ltd. Phone +61 02 8877 7400 www.miltenyibiotec.com macs@miltenyibiotec.com.au Miltenyi Biotec B.V. (Benelux) macs@miltenyibiotec.nl **Customer service Netherlands** Phone 0800 4020120 **Customer service Belgium** Phone 0800 94016 **Customer service Luxembourg** Phone 800 24971

Miltenyi Biotec Trading (Shanghai) Co., Ltd. (P.R. China) Phone +86 21 6235 1005 macs@miltenyibiotec.com.cn

Miltenyi Biotec SAS (France) Phone +33 1 56 98 16 16 macs@miltenyibiotec.fr

Miltenyi Biotec S.r.l. (Italy) Phone +39 051 646 0411 macs@miltenyibiotec.it

Miltenyi Biotec K.K. (Japan) Phone +81 3 5646 8910 macs@miltenyibiotec.jp

Miltenyi Biotec Asia Pacific Pte. Ltd. (Singapore) Phone +65 6238 8183 macs@miltenvibiotec.com.sg

Miltenyi Biotec S.L. (Spain) Phone +34 91 512 12 90 macs@miltenyibiotec.es

Miltenyi Biotec Ltd. (UK) Phone +44 1483 799 800 macs@miltenyibiotec.co.uk