

PRODUCT DATA SHEET

ANTI-HUMAN GRANZYME K MONOCLONAL ANTIBODY

PRODUCT INFORMATION

Catalog Number:	GM-0202	Clone:	GM-24C3
Description:	purified monoclonal mouse antibody	Specificity:	anti-human Granzyme K (GrK; granzyme-3; NK-tryptase-2)
Isotype:	IgG2b/kappa	Purification:	Protein G
Storage:	short term: 2°C – 8°C; long term: -20°C (avoid repeated freezing and thawing)	Buffer:	phosphate buffered saline, pH 7.2
Immunogen:	genetic immunization with cDNA encoding human Granzyme K	Selection:	based on recognition of the complete native protein expressed on transfected mammalian cells

WORKING DILUTIONS

Flow cytometry:	1.2 µg/10 ⁶ cells	CELISA:	1:200 – 1:400
ELISA:	1:200 – 1:400		

For each application a titration should be performed to determine the optimal concentration.

SPECIFICITY TESTING BY FLOW CYTOMETRY

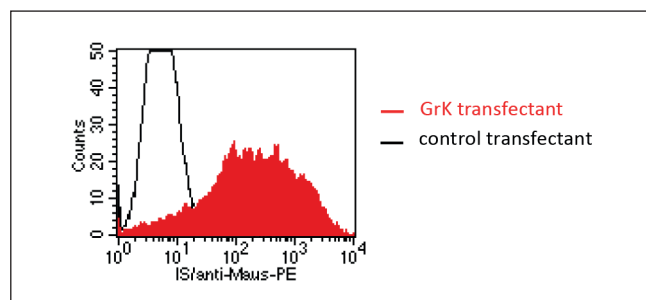


Fig. 1: FACS analysis of BOSC23 cells using GM-24C3 Cat.# GM-0202. BOSC23 cells were transiently transfected with an expression vector encoding either Granzyme K (red curve) or an irrelevant protein (control transfectant: black curve). Binding of GM-24C3 was detected with a PE-conjugated secondary antibody. A positive signal was obtained only with Granzyme K transfected cells.

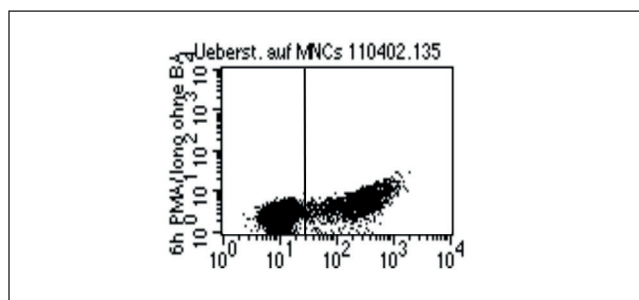


Fig. 2: Intracellular detection of granzyme B in human PBMC. FACS analysis of human PBMC using hybridoma supernatant of GM-24C3. PBMC were cultivated in the presence of phorbol ester and ionomycin subsequently fixed and permeabilised. Binding of GM-24C3 was detected with a FITC-conjugated secondary antibody.

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ANTIBODY CROSS-REACTIVITY WITH MEMBERS OF THE GRANZYME FAMILY

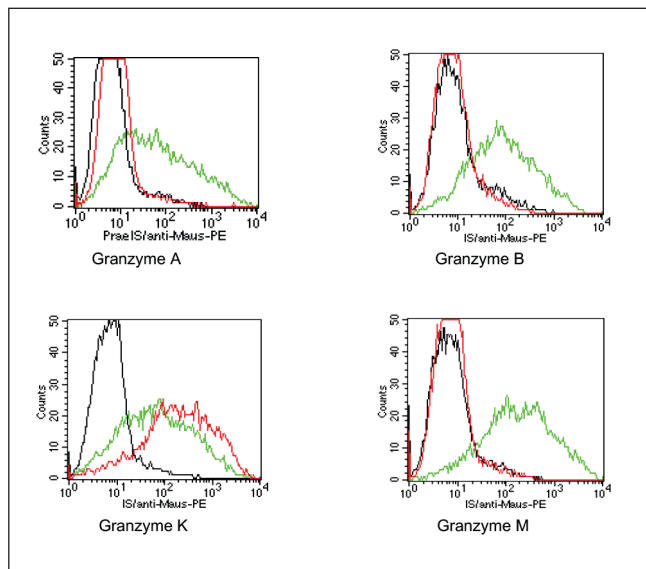


Fig. 3: BOSC cells were transiently transfected with expression vectors for Granzyme A, B, K, or M. Expression of the constructs was tested with an anti-myc monoclonal antibody (green curves), an irrelevant monoclonal antibody served as negative control (black curves). For specificity testing, GM-24C3 hybridoma supernatant was tested on all transfectants. A positive signal was obtained only with Granzyme K transfected cells (red curves).

BACKGROUND

Granzyme K (GrK) belongs to a family of trypsin-like serine proteases localised in the cytotoxic granules of activated T cells and NK cells. It encodes a 28 kDa serine protease whose gene is located on chromosome 5q11-12 close to the granzyme A-encoding gene. Like granzyme A, it has a trypsin-like specificity cleaving at the basic residues arginine and lysine. Granzyme K triggers rapid cell death independently of caspase activation with single-stranded DNA nicks and is primarily expressed in thymus, lung, spleen and peripheral blood leukocytes.

REFERENCES

1. **Bade B, Lohrmann J, ten Brinke A, Wolbink AM, Wolbink GJ, ten Berge IJM, Virchow JC Jr, Luttmann W and CE Hack (2005).** Detection of soluble human granzyme K in vitro and in vivo. *Eur. J. Immunol.* (10): 2940-2948.
2. **Bade B, Boettcher HE, Lohrmann J, Hink-Schauer C, Bratke K, Jenne DE, Virchow JC Jr and W. Luttmann (2005).** Differential expression of the granzymes A, K and M and perforin in human peripheral blood lymphocytes. *Int Immunol.* (11): 1419-1428.

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Page 2 / 2