

Development of Antibodies Specific for NKG2 Family Members

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INTRODUCTION

NKG2A, NKG2C and NKG2E/H are members of the NKG2 family of type II transmembrane receptors, which have closely-related extracellular C-type lectin domains. These proteins are expressed in NK cells and some T cell subsets. NKG2E and NKG2H are splice variants encoded by the same gene, whereas NKG2A and NKG2C are encoded by different genes. NKG2A, NKG2C and NKG2E/H all require association with CD94 for cell-surface expression and functional activity. Although members of the NKG2 family have similar extracellular domains, their cytoplasmic domains are functionally distinct. The NKG2A/CD94 complex delivers an inhibitory signal through association with ITIM motifs in the cytoplasm. The NKG2C/CD94 and NKG2E/CD94 complexes associate with the ITAM-bearing DAP12 adapter protein and deliver an activating signal upon ligand binding.

Due to these functional differences, an antibody that is specific for NKG2A can help to distinguish between the expression of the inhibitory NKG2A receptor and the activating NKG2E receptor. We have developed and characterized panels of monoclonal antibodies (mAbs) that recognize members of the NKG2 family. Immunization of Balb/c mice with Ba/F3 NKG2A + CD94 transfectant generated one mAb (Clone # 131411) that specifically recognizes NKG2A by flow cytometry. The same fusion yielded several mAbs that cross-react with NKG2C, as well as mAbs specific for CD94. We show that anti-human NKG2A (Clone # 131411) specifically recognizes NKG2A transfectant cells, and not NKG2C, NKG2E/H, or CD94. In contrast, another commercially available clone (Clone # Z199) stained both NKG2A and NKG2E/H-expressing Ba/F3 transfectants, but was not cross-reactive with NKG2C or CD94.

MATERIALS & METHODS

Alignment of the amino acid sequences of NKG2A, C, E and H.

The transmembrane regions are underlined. Unique residues in the extracellular domain are highlighted in blue. NKG2E and NKG2H are splicing variants encoded by a single gene. The NKG2E/H transfectant includes the first 223 amino acids of NKG2E/H with a 9 amino acid segment from the C-terminus of NKG2C (shown in red) to promote surface expression. The cell lines transfected with an NKG2 family member were also co-transfected with CD94.

E/H transfect	NKQRGTFSEVSLAQDPKPKQPKGNKSSISGTEQEIFQVELNLQNASLNHQGIDKIYD
NKG2C	MSKQRGTFSEVSLAQDPKPKQPKGNKSSISGTEQEIFQVELNLQNPNSLNHQGIDKIYD
NKG2A	MDNQGVVYSDLNLPNPKRQQRKPKGNKSSILATEQEIFYAEINLQKASQDFQGNKDYH
E/H transfect	CQGLLPPEKLTAEVLGIIICIVLMATVLTIVLIP---FLEQNNSSPNTRTQKARPCGHC
NKG2C	CQGLLPPEKLTAEVLGIIICIVLMATVLTIVLIP---FLEQNNSSPNTRTQKARHCGHC
NKG2A	CKDLPSAPEKLVIGIIGIICILMAS-VVTIVVIPSTLIQRHNNSSLNTRTQKARHCGHC
E/H transfect	PEEWITYSNSCYIIGKERRTWEESLQACASKNSSLLSIDNEEEMKFLASILPSSWIGVF
NKG2C	PEEWITYSNSCYIIGKERRTWEESLLACTSKNSS-LLSIDNEEEMKFLASILPSSWIGVF
NKG2A	PEEWITYSNSCYIIGKERRTWEESLLACTSKNSS-LLSIDNEEEMKFLSISIPSSWIGVF
E/H transfect	RNSSHPWVTINGLAFKHEIKDSDHAERNCAMLHVRGLISDQCGSSMIYHCKHKL
NKG2C	RNSSHPWVTINGLAFKHEIKDSDNAELNCAVLQVNRKLSAQCGSSMIYHCKHKL
NKG2A	RNSSHPWVTINGLAFKHEIKDSDNAELNCAVLQVNRKLSAQCGSSITTYHCKHKL
NKG2H	RNSSHPWVTINGLAFKHEIKDSDHAERNCAMLHVRGLISDQCGSSRIIVSISFRIKALE
NKG2E	RNSSHPWVTINGLAFKHEIKDSDHAERNCAMLHVRGLISDQCGSSRIIRRGFIMLTRLV
NKG2H	LAVHQIKFYICSNRNDIMIA
NKG2E	LNS-----

Human PBMC preparation:

A single cell suspension of PBMC was prepared on a Ficoll®-Hypaque density gradient and resuspended in 1X MagCollect™ Buffer.

Isolation of human NK cells:

Human NK cells were enriched using R&D Systems MagCollect Human NK Cell Isolation Kit (Catalog # MAGH109). Briefly, 50 million human PBMC were incubated with MagCollect NK cell depletion cocktail for 15 minutes at 4° C, followed by the addition of streptavidin-conjugated magnetic ferrofluid for another 15 minutes. Cells were subsequently placed on a magnet for 6 minutes and NK cells in the supernatant were collected. Isolated cells were characterized by flow cytometry. Isolated cells were ~80% CD56⁺.

All other methods and materials used are detailed with the data.

MagCollect is a trademark of R&D Systems.
Ficoll is a registered trademark of GE Healthcare Bio-Sciences.

RESULTS

CLONE #	ISOTYPE	SPECIFICITY
131411	IgG2A	NKG2A
131403	IgG2B	NKG2A/NKG2C
131407	IgG1	NKG2A/NKG2C
131408	IgG2A	NKG2A/NKG2C
131410	IgG1	NKG2A/NKG2C
131415	IgG2B	NKG2A/NKG2C
131417	IgG1	NKG2A/NKG2C
131405	IgG2A	CD94
131412	IgG1	CD94
131416	IgG1	CD94
134522	IgG2B	NKG2C
134534	IgG2B	NKG2C
134537	IgG2B	NKG2C
134557	IgG2B	NKG2C
134571	IgG2A	NKG2C
134572	IgG1	NKG2C
134591	IgG1	NKG2C

TABLE 1. Antibody Clones that Recognize NKG2A, NKG2C, and CD94.

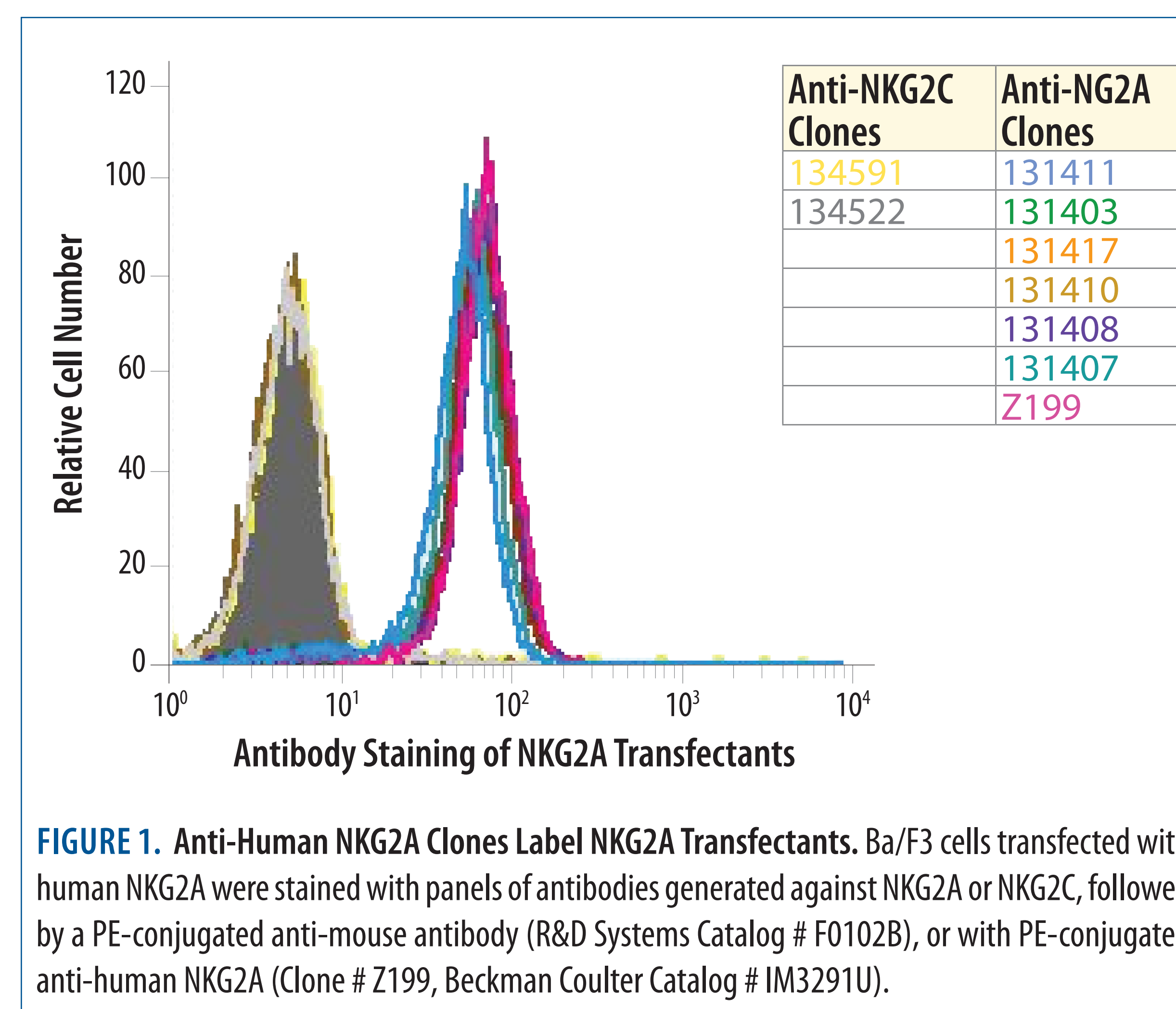


FIGURE 1. Anti-Human NKG2A Clones Label NKG2A Transfectants. Ba/F3 cells transfected with human NKG2A were stained with panels of antibodies generated against NKG2A or NKG2C, followed by a PE-conjugated anti-mouse antibody (R&D Systems Catalog # F0102B), or with PE-conjugated anti-human NKG2A (Clone # Z199, Beckman Coulter Catalog # IM3291U).

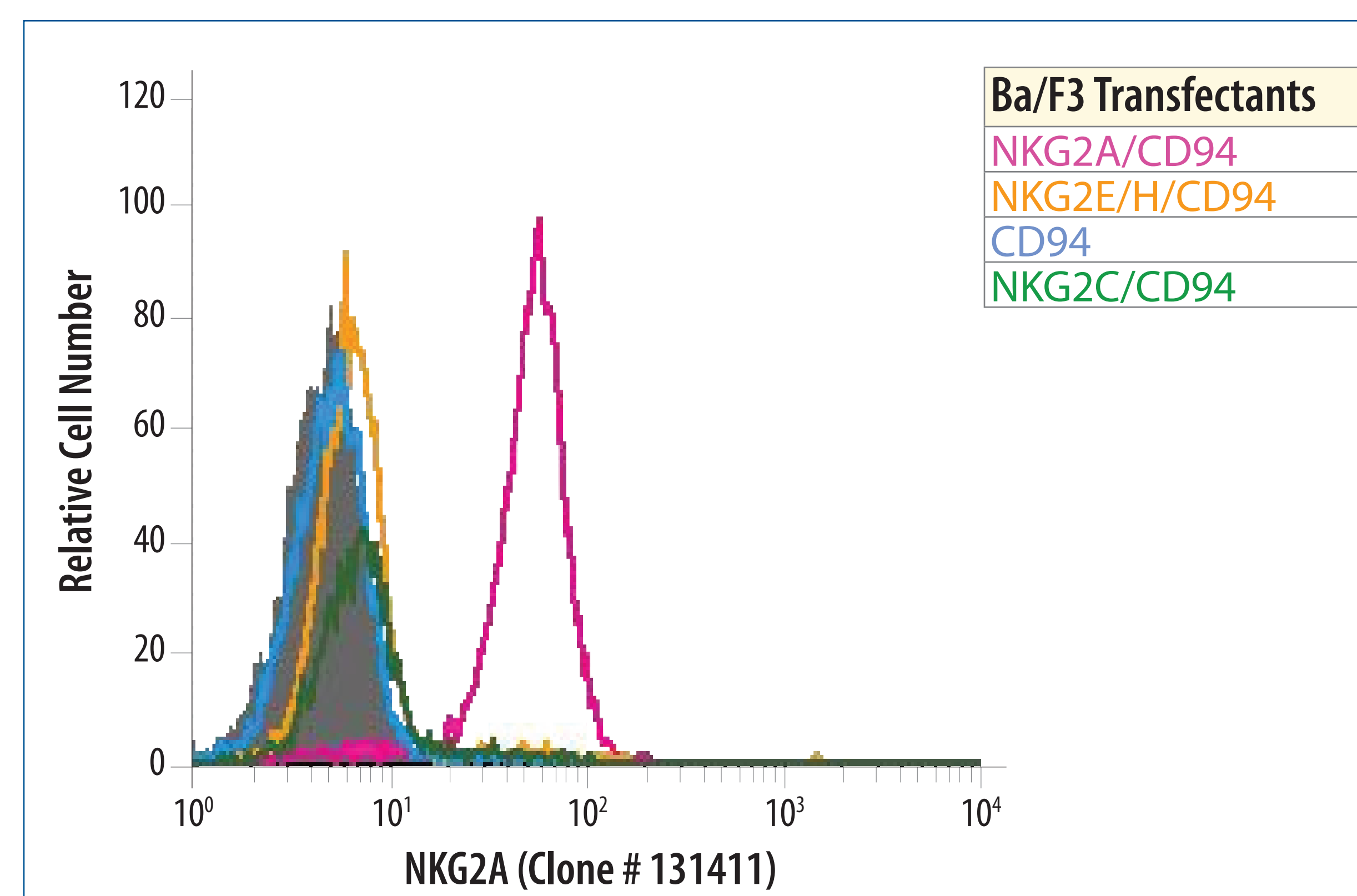


FIGURE 2. Anti-Human NKG2A (Clone # 131411) is Specific for Human NKG2A. Ba/F3 cells transfected with human NKG2 family members and/or CD94 were stained with anti-human NKG2A (Clone # 131411, R&D Systems Catalog # MAB1059) followed by a PE-conjugated anti-mouse antibody (R&D Systems Catalog # F0102B).

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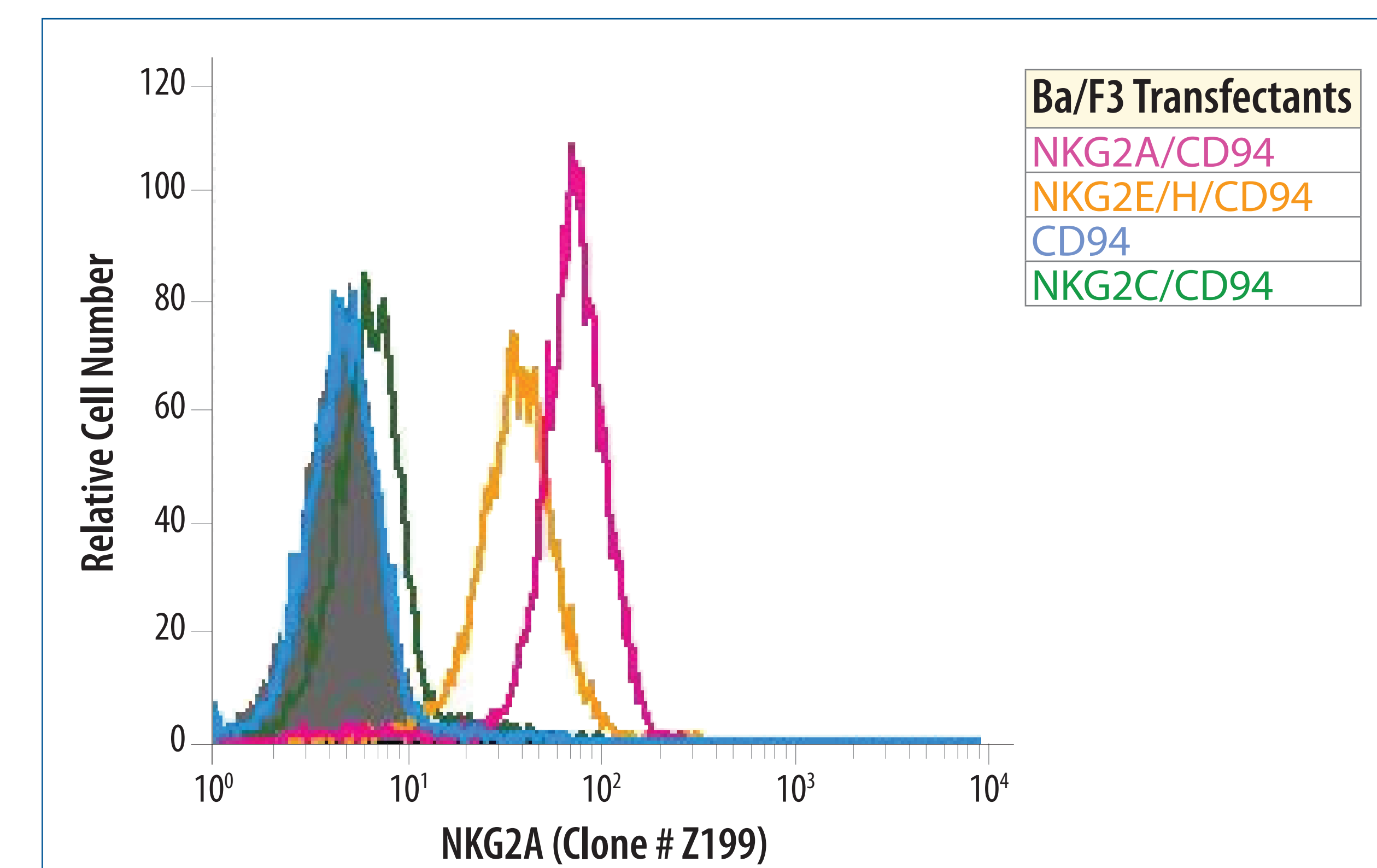


FIGURE 3. Anti-human NKG2A (Clone # Z199) cross-reacts with NKG2E/H. Ba/F3 cells transfected with human NKG2 family members and/or CD94 were stained with PE conjugated anti-human NKG2A (Clone # Z199, Beckman Coulter Catalog # IM3291U). The clone recognizes both NKG2A/CD94 and NKG2E/H/CD94 transfectants.

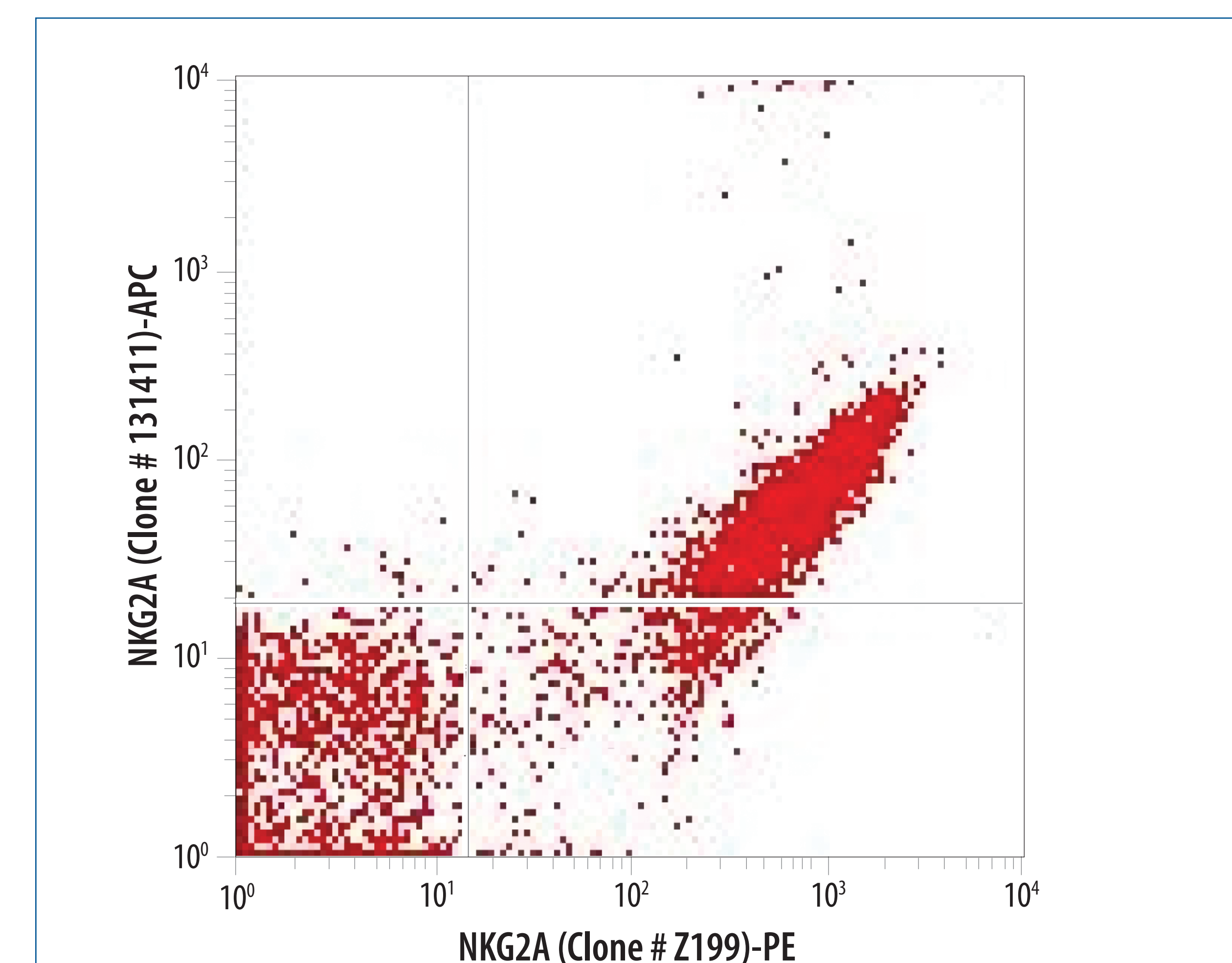


FIGURE 4. Two NKG2A Clones Assessed by Flow Cytometry. Enriched human peripheral blood NK cells (R&D Systems MagCollect Human NK Cell Isolation Kit, Catalog # MAGH109) were stained with PE-conjugated anti-human NKG2A (Clone # Z199, Beckman Coulter Catalog # IM3291U) and APC-conjugated anti-human NKG2A (Clone # 131411, R&D Systems Catalog # FAB1059A). Quadrant markers were set based on isotype control staining.

SUMMARY

- Specificity must be carefully assessed when selecting monoclonal antibodies for NKG2 family members.
- Anti-human NKG2A (Clone # 131411) is specific for NKG2A/CD94 transfectants.
- Anti-human NKG2A (Clone # Z199) detects both NKG2A/CD94 and NKG2E/H/CD94 transfectants.

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