Max-Planck-Innovation

Technology Offer

Anti-mouse and anti-rabbit IgG secondary VHH single domain antibodies optimized for all immuno-labeling applications

File no.: MI-0707-5419-LI-ZE

Max-Planck-Innovation GmbH
Amalienstr. 33
80799 Munich
Germany

Phone: +49 (89) 29 09 19 - 0
Fax: +49 (89) 29 09 19 - 99
info@max-planck-innovation.de
www.max-planck-innovation.de

Contact:
Dr. Dieter Link
Tel.: 089 / 290919-28
link@max-planck-innovation.de

Background

Anti-IgG secondary antibodies are major tools for the detection or immobilization of primary antibodies in approaches such as western blotting, ELISA, Co-IP and immunofluorescence. Polyclonal anti-IgG secondary antibodies are produced in animals, such as mouse, rabbit, rat, and goat and are directed against the IgG classes of these animals. Thus, the generation of such secondary antibodies requires the keeping and use of a large number of animals, which is not only costly but also a major ethical problem. Furthermore, a prerequisite for the use of these anti-IgG secondary antibodies is a labour-intensive quality control, since each new batch of serum obtained from the animals is a heterogeneous mixture, which requires depletion of non-specific and cross-reacting antibodies.

Technology

Scientists from the Max-Planck Institute of Biophysical Chemistry in Göttingen have developed anti-IgG secondary VHH single domain antibodies (sdAbs) that outperform commercially available anti-IgG secondary antibodies with regard to sensitivity and specificity and thus offer a sustainable alternative (Figure 1) (ref. 1). A major advantage of the new anti-IgG VHH sdAbs is their easy, cost-effective and animal-friendly production at large scale by expression in *E. coli*. VHH sdAbs are originally derived from camelid heavy chain antibodies and have a molecular weight of approximately 13 kDa. The VHH sdAbs developed here recognize all mouse IgG subclasses and the sole class of rabbit IgG. Due to their recombinant nature, they can be easily fused to affinity tags or reporter enzymes, such as peroxidases.

Moreover, the VHH sdAbs can be easily linked to all fluorophores via maleimide chemistry and can even be coupled to up to three dyes (ref. 2). The site-specific labeling with multiple fluorophores creates bright imaging reagents suitable for high-resolution confocal and super-resolution microscopy. Since VHH sdAbs are much smaller in size than secondary antibodies they can place fluorophores closer than 2 nm to the primary antibody and thus are perfectly suited for super-resolution imaging. Our scientists have rigorously tested and optimized these VHH sdAbs for all common immuno-labeling approaches. Due to their monovalent nature, these VHH sdAbs do not crosslink primary antibodies and thus allow simpler one-step immuno-staining protocols (pre-incubation of primary antibody with VHH sdAbs). This even enables multi-target localization with primary IgG antibodies from the same species and of the same IgG subclass (Figure 2) (ref. 1).

Taken together, this technology has the potential to replace conventional anti-mouse or anti-rabbit IgG secondary antibodies and it overcomes the lack of signal strength in available alternatives. Currently, we are looking for partners who are interested in in-licensing this technology.

Patent Information: A European priority application has been filed on September 2017.

Reference: 1) Pleiner T, Bates M. and Görlich D., 2017. Available online at bioRxiv: https://doi.org/10.1101/209742
2) Pleiner T. et al., 2015. eLife 2015;4:e11349

Figures:

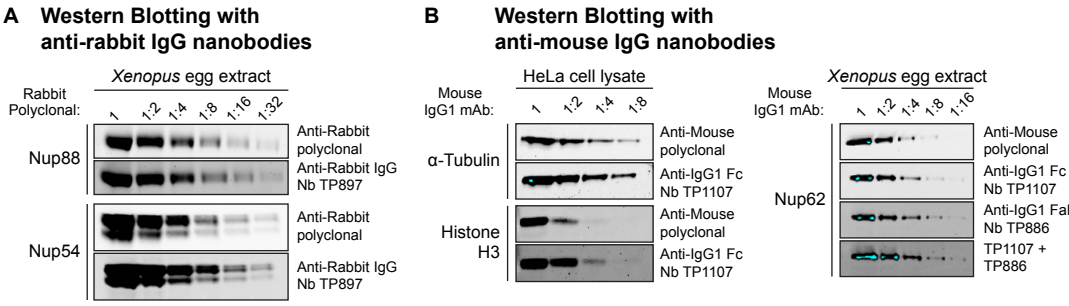

Figure 1. Western blotting with infrared dye labeled anti-IgG nanobodies.
(**A**) A twofold dilution series of Xenopus laevis egg extract was analyzed by SDS-PAGE and Western Blotting. The indicated rabbit polyclonal antibodies were used to detect nucleoporins (Nups). These primary antibodies were then decorated either via IRDye 800-labeled goat anti-rabbit polyclonal IgG (1:5,000; LI-COR Biosciences, USA) or anti-rabbit IgG nanobody TP897 (10 nM). Blots were analyzed with an Odyssey Infrared Imaging System (LI-COR Biosciences, USA).
(**B**) (Left panel) A twofold dilution series of HeLa cell lysate was analyzed by SDS-PAGE and Western Blotting. The indicated mouse IgG1 mAbs were decorated either via IRDye 800-labeled goat anti-mouse polyclonal IgG (1:1,340, 5 nM, LI-COR Biosciences, USA) or anti-mouse IgG1 Fc nanobody TP1107 (5 nM). (Right panel) A twofold dilution series of Xenopus egg extract was blotted and probed with anti-Nup62 mouse IgG1 mAb A225. It was then detected either via IRDye 800-labeled goat anti-mouse polyclonal IgG (5 nM), anti-mouse IgG1 Fc nanobody TP1107 (5 nM), anti-mouse IgG1 Fab nanobody TP886 (5 nM), anti-mouse kappa chain nanobody TP1170 (2.5 nM), a combination of TP1107 and TP886 or TP1107 and TP1170. Blue pixels indicate signal saturation.

(Please note: Nanobody is a registered trademark of Ablynx.)

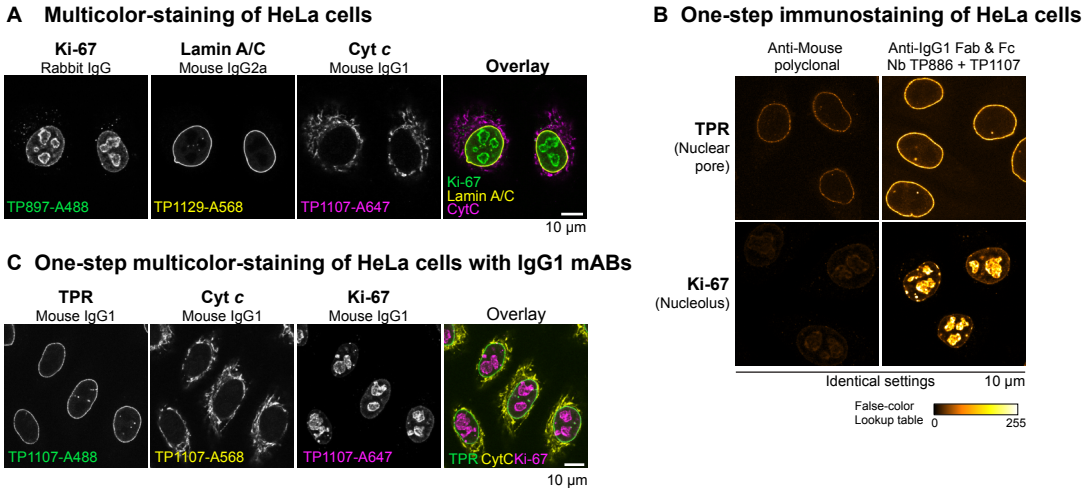

Figure 2. Multicolor and one-step immunostaining of HeLa cells with anti-IgG nanobodies.
(**A**) Multicolor-staining of HeLa cells. HeLa cells were incubated with the indicated mouse IgG1, mouse IgG2a or rabbit IgG antibodies. These primary antibodies were detected via anti-mouse IgG1 Fc nanobody TP1107, anti-mouse IgG2a Fc nanobody TP1129 or anti-rabbit IgG nanobody TP897, respectively, labeled with the indicated Alexa dyes.
(**B**) The indicated mouse IgG1 mAbs were pre-incubated with an equal amount of Alexa 488-labeled goat anti-mouse secondary antibody or a combination of anti-mouse IgG1 Fab nanobody TP886 and anti-mouse IgG1 Fc nanobody TP1107.The resulting mixes were then applied to fixed and blocked Hela cells. After washing, the cells were directly mounted for imaging. For every primary antibody, images were acquired under identical settings and pixel intensities are represented via a false-color lookup table.
(**C**) Multicolor-staining of HeLa cells with mouse IgG1 subclass mAbs. The indicated mouse IgG1 mAbs were separately pre-incubated with Alexa 488, Alexa 568 or Alexa 647-coupled anti-mouse IgG1 Fc nanobody TP1107 and then mixed before staining HeLa cells in a single step. Washed cells were directly mounted for imaging.

(Please note: Nanobody is a registered trademark of Ablynx.)