

Product datasheet

Mouse IgG Library Primer Set

Short overview

Cat. No.	F2010
Quantity	2x25 primers

Product description

Formulation	Ready-to-use for common PCR (conc. 10 pmol/μl in 10 mM Tris-HCl pH 8, 1 mM EDTA)
Specificity	PCR amplification (Set 1) and cloning (Set 2) of mouse IgG heavy and light chain variable domain coding regions
Stability	Minimum 1 year at -20°C
Product description	Generation of large repertoires of rearranged immunoglobulin variable domain coding regions for the construction of mouse IgG scFv-antibody libraries. Amplification of immunoglobulin variable gene fragments from single B cell clones.
Note	Volume: 20 to 240 μl ready-to-use primer
Purification	HPLC or HPSF purification
Storage	-20°C
Intended use	Research use only

Background

Introduction Many monoclonal antibodies of mouse origin are valuable diagnostic agents. Their production by classical hybridoma techniques is frequently limited by the instability of cell lines, low antibody yields and the limitations of immunizing mice with toxic antigens. A promising alternative to the hybridoma technology is the production of recombinant antibodies.

Pioneering work of the last decade showed that it is possible to amplify rearranged immunoglobulin genes from B-lymphocytes, to insert them into different vectors, and to express them in bacteria, yeast, insect, mammalian or plant cells. Moreover, the randomized combination of cloned heavy and light chain immunoglobulin gene fragments allowed the construction of mouse antibody libraries. These libraries enable the isolation of specific antibodies against particular antigens by phage display techniques. One prerequisite for generating highly diversified mouse antibody libraries, however, is the development of PCR primers capable of amplifying all rearranged immunoglobulin genes. In immunoglobulin repertoire library cloning, the homology between a particular primer sequence and its target template, as well as the diversity of a primer pool are the two most important parameters which determine the cloning efficiency and the size of a resulting repertoire library.

PROGEN's mouse IgG library primer set allows the amplification of rearranged mouse immunoglobulin genes of individual B cell clones as well as of larger B cell populations for the construction of mouse scFv antibody libraries.

PCR Primer Design The PCR oligonucleotide primers were designed for the amplification of mouse rearranged IgG variable domain coding regions in a two step PCR. The first set of PCR primers (Set 1) leads to the amplification of the antibody genes from a lymphocyte cDNA source. The primer design started with the analysis of mouse antibody sequences from databases. Similar sequences were grouped and putative primer sequences were drawn from each group. All putative primer sequences were then compared against all database sequences and the best-fitting primer sequences were collected. The process was repeated until all the database sequences were covered.

To clone the amplified immunoglobulin gene fragments into expression vectors, a second set of homologous primers (Set 2) containing restriction endonucleases sites was designed. These primers add the recognition sequences of Nco I (5') and Hind III (3') to the amplified heavy chain gene fragments and Mlu I (5') and Not I (3') to the amplified light chain gene fragments. The restriction enzymes have been selected, (a) because of their low probability to cut within mouse variable heavy and light chain coding regions. Additionally, (b) they produce overlaps of 4 nucleotides or more leading to optimal cloning efficiency. The enzymes (c) are not depending on methylation and (d) their efficiency in recommended double digestions is more than 90%. Finally, they were selected for cloning the amplified gene fragments into pSEX or pOPE vector derivatives (Breitling et al., 1991; Duebel et al., 1993).

Material Required PCR reagents and equipment

Preparation of Reagents The mRNA and cDNA from lymphocytes should be prepared as described in standard protocols.

PCR-Setup We propose to use a commercial PCR kit containing a proof-reading polymerase and to set up the reactions as recommended by the manufacturer. Most PCRs are carried out in a 50 microliter reaction. Within this volume, we recommend to use 50-100 ng of cDNA and 25 pmol (2.5 microliter) of each primer (10 pmol/microliter). With the first set of primers 25 separate reactions should be performed. Each variable heavy chain forward primer (1A-L) has to be combined with the constant region IgG reverse primer (1M). Analogous, each kappa- and lambda-light chain forward primer (1N-W, 1Y) has to be combined with the corresponding constant region reverse primer (1X or 1Z), respectively. The reaction mixtures accomplished with dNTPs, buffer and polymerase should be subjected to 30 PCR cycles using an annealing temperature of 55 degree Celsius. Analyse the reaction mixtures by electrophoresis using a 1.5% agarose gel. Amplified DNA fragments of approx. 380 to 400 bp should be gel purified and subjected to an analogous second PCR using the second primer set to introduce the restriction endonuclease sites.

Product images



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References

Publication	Species	Application
<p>Dolgikh, V. V. et al. Construction of scFv Antibodies against the Outer Loops of the Microsporidium Nosema bombycis ATP/ADP-Transporters and Selection of the Fragment Efficiently Inhibiting Parasite Growth. Int. J. Mol. Sci. 23, (2022).</p>		
<p>Boutajangout, A. et al. Passive Immunization With a Novel Monoclonal Anti-PrP Antibody TW1 in an Alzheimerâ€™s Mouse Model With Tau Pathology. Front. Aging Neurosci. 13, 1â€“14 (2021).</p>		
<p>Rajput, R. et al. Diagnostic potential of recombinant scFv antibodies generated against hemagglutinin protein of influenza A virus. Front. Immunol. 6, 1â€“9 (2015).</p>		
<p>Pauly, D. et al. A novel antibody against human properdin inhibits the alternative complement system and specifically detects properdin from blood samples. PLoS One 9, (2014).</p>		
<p>Zhou, H., Fisher, R. J. & Papas, T. S. Optimization of primer sequences for mouse scFv repertoire display library construction. Nucleic Acids Res. 22, 888â€“889 (1994).</p>		