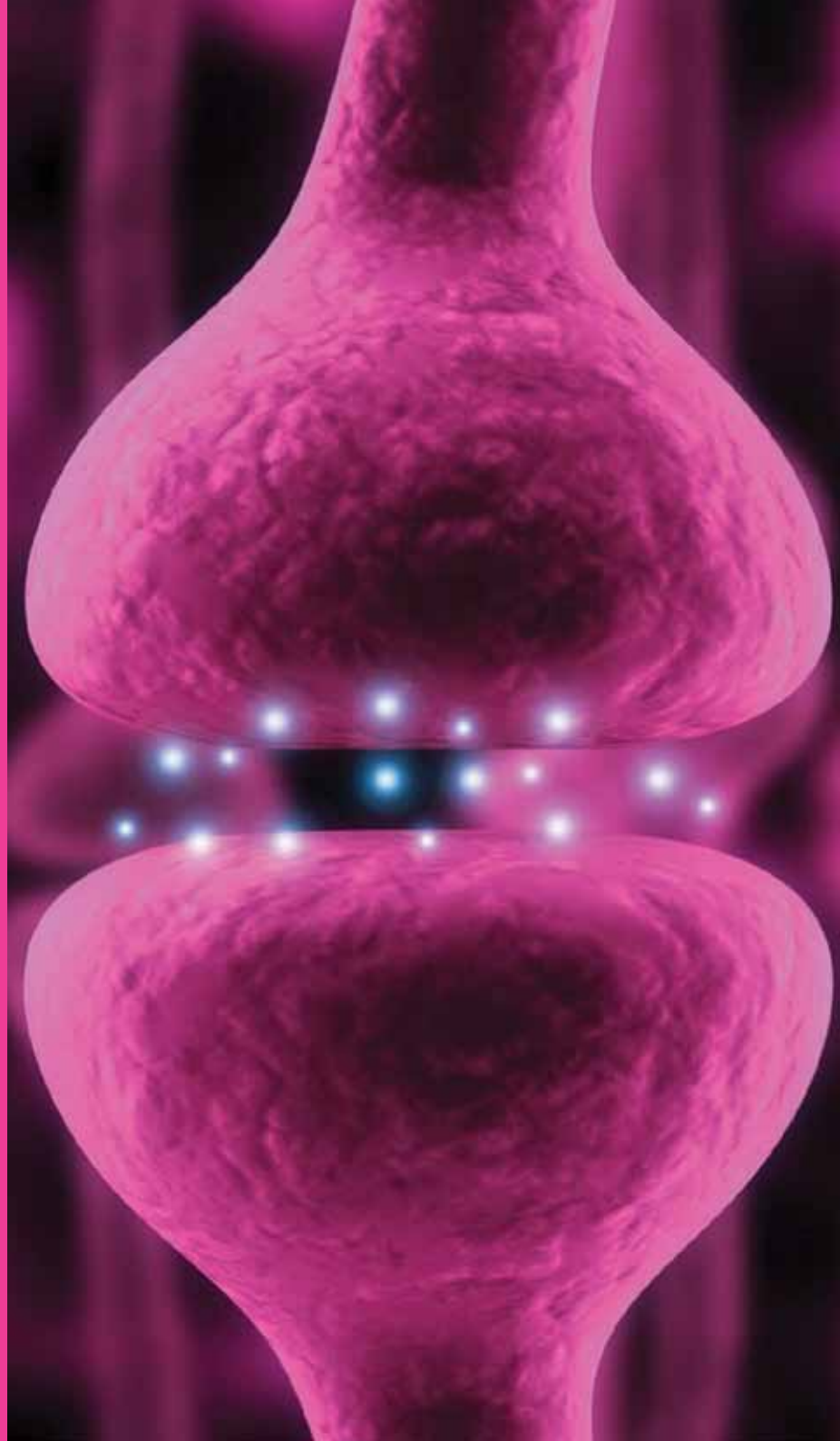


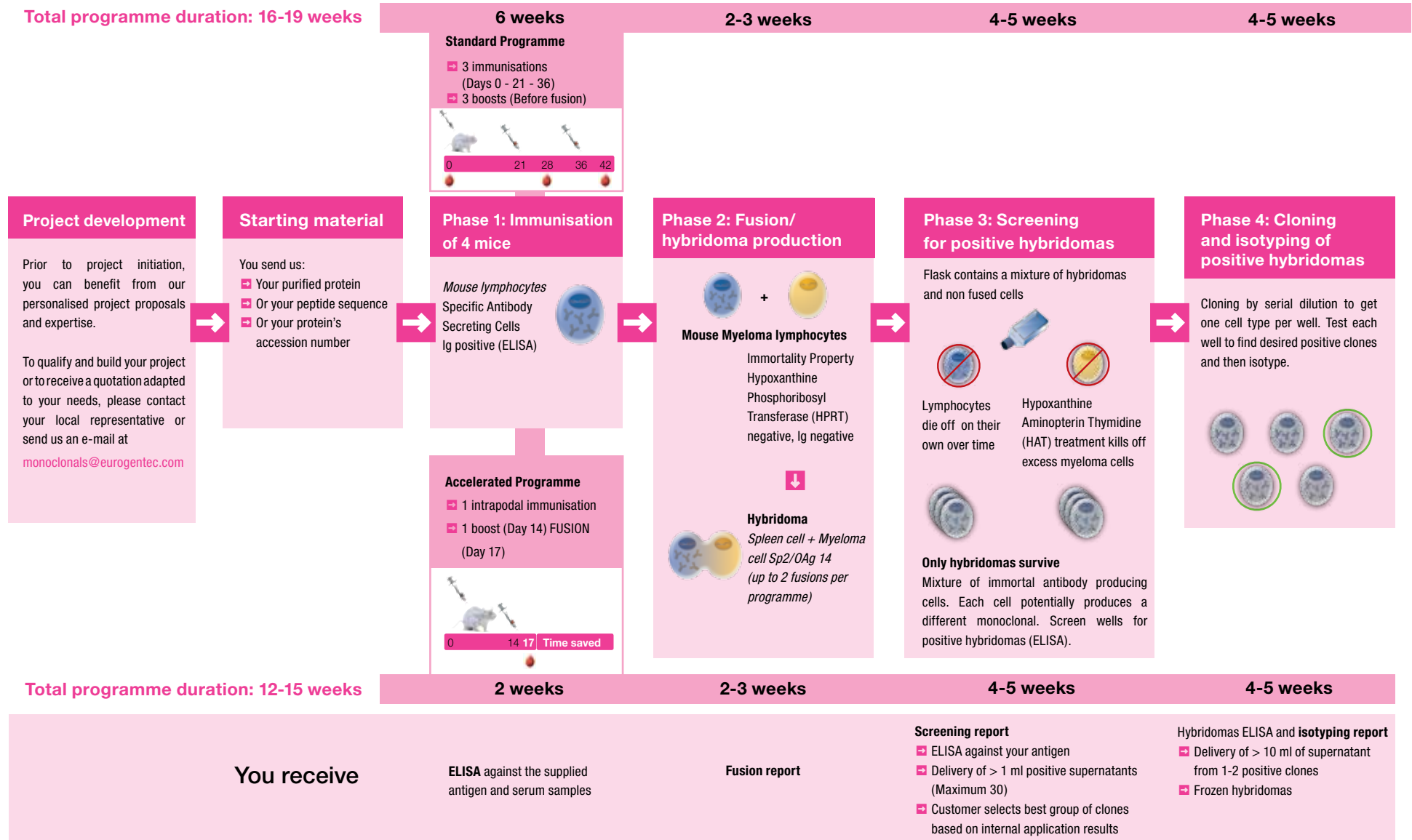
# 3

## 3.2 Custom Monoclonal

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# Mouse monoclonal antibody development



## Options

- Additional clones can be selected during phase 4
- Selection of the right Mo-Ab couple for sandwich assay development

## Additional Quality Control

- Specificity via Western Blot
- Affinity via Surface Plasmon Resonance (Biacore)

### Mouse monoclonal phases

Phase	Reference
Phase 1 - Immunisation	AS-ACMC-IM (X4) 4 mice or AS-ACMC-IMFAST (X4) 4 mice-Fast protocol
Phase 2 - Fusion & hybridoma production	AS-ACMC-FU08
Phase 3 - Screening	AS-ACMC-AN08S
Phase 4 - Cloning & Isotyping	AS-ACMC-CIFH (1-2 clones)
Phase 4 - Additional clone	AS-ACMC-CIAH

If no positive hybridoma is obtained after phase 3, only phase 1 will be charged.

### Additional quality control

Additional quality control	Reference
Western blot	AS-ACMC-QCWB
Antibody Affinity evaluation with Biacore 2000	AS-ACMC-QCAFFI
FACS analysis	AS-ACMC-QCFACS

## In vitro Monoclonals expression service

Eurogentec is happy to provide *in vitro* Monoclonals expression service of milligrams to gram quantities of mouse, rat, humanized or fully human IgG (in flasks, roller or MiniPerm®):

- Using state of the art technologies providing a better antibody production reproducibility and purity compared to the ascite production, and for the same cost.
- No specificity of the clone required, ensuring a perfect confidentiality

### The project steps are the following:

- Your clone:** You provide us with the clone and a technical data sheet mentioning the culture medium and the antibody isotype. The hybridoma may also be adapted to different culture media (IgG-depleted serum medium, medium without serum...)
- Quotation:** For quantities > 100 mg, we propose a productivity evaluation in smaller volumes. You will receive a report indicating the best-obtained productivity and the quotation for the whole production. You may also receive the produced antibody to make an evaluation prior to the production phase.
- In vitro production:** This phase starts on volumes from 1 up to >100 liters of culture, allowing the production of up to several grams of antibodies.
- Production follow-up:** IgG (mouse, rat, human) titration kits are used to follow the antibody production in the culture supernatant allowing a quick estimation of the supernatant volume to be used to reach the assigned quantity.

5. **Purification:** Up to 15-fold concentration of the supernatant can be made via ultrafiltration before starting the monoclonal antibody purification. This purification is done by affinity chromatography on Protein A or Protein G depending on the clone's origin or isotype.

- For IgM, the purification is done through ion exchange chromatography.
- Optionally, the produced antibodies may be purified in Endofree conditions.
- Antibodies purity may be enhanced via ion exchange chromatography, which is also eliminating the potential endotoxins. Endofree Monoclonals have an endotoxin level below 1 EU/mg. Your *in vitro* monoclonals are ready to be delivered under the concentration and buffering condition you specified.

6. **Quality Control:** With your Monoclonal antibody you also receive the quality control file including the SDS-PAGE control and total protein concentration (Lowry). You may also receive a LAL (Limulus Amebocyte Lysate) test of bacterial endotoxin level.

Note: In rare occasion where *in vitro* production is too difficult, we may produce your monoclonal antibody in ascites (from 5 mice/hybridoma).

**Please contact us ([info@eurogentec.com](mailto:info@eurogentec.com)) for more information on this service**

### Custom mouse monoclonal antibody

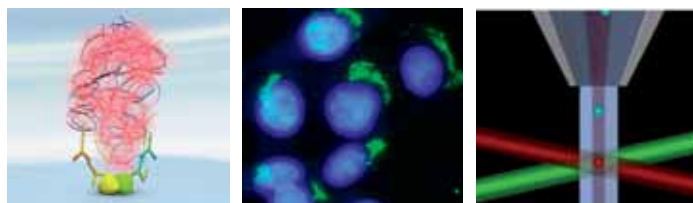
Description	Reference
Monoclonal antibody production, <i>in vitro</i> , roller bottle, 4 weeks	AS-ACMC-ROLLB
Monoclonal antibody production, <i>in vitro</i> , CL1000, 4 weeks	AS-ACMC-CL1000

# Mouse monoclonal antibody additional services

## Antibody labelling

Depending on your application (DuoLink®, *in vivo* / *in vitro* localisation, FACS ...), your antibodies can be labelled with:

- ▣ HiLyte Fluor dyes
- ▣ Phycoerythrine
- ▣ Rhodamine
- ▣ FITC
- ▣ Alexa®
- ▣ BODIPY®
- ▣ Cy®3, Cy5®
- ▣ Texas Red®
- ▣ Horse Radish Peroxydase (HRP)
- ▣ Alkaline Phosphatase
- ▣ Biotin
- ▣ Digoxigenin...



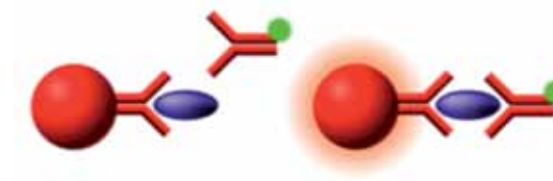
## Fragmentation

Your antibodies are selectively cleaved into Fab, F(ab')<sub>2</sub> and Fc fragments.

## Assay development

**ELISA:** Your antibodies can be coated on plates.

**Luminex:** Your antibodies can be coated on microspheres.



**Purification:** Your antibodies can be coated on beads for affinity purification, co-immunoprecipitation...

**Epitope Mapping:** Your antibodies can be tested against 13 aa long peptides (see Custom Peptides chapter)

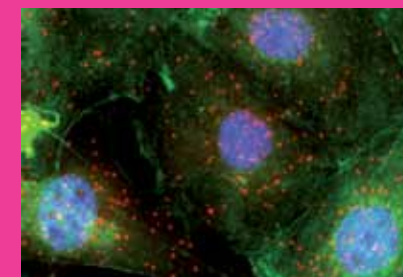
## Cell banking

You can ask us to freeze cells as a backup for long term storage.

To qualify and build your project or to receive a quotation adapted to your needs, please contact your local representative or send us an e-mail at [monoclonals@eurogentec.com](mailto:monoclonals@eurogentec.com)

## Duolink®

Discover more about proteins and protein interactions



- ▣ Visualize and Quantify Protein interactions
- ▣ Localize your target with single molecule resolution
- ▣ See page 261

# Magnetic beads coupling services

Eurogentec has joined forces with a state-of-the-art bead manufacturer, to offer you antibody-magnetic bead complexes, ready-to-go for your application.

## Save time and money

Don't lose your precious time setting up coupling protocols. Eurogentec does it for you, at an affordable price.

## Get the best results

Eurogentec has conducted many investigations to choose the best beads available, and select the most efficient coupling protocol.

## Focus on your target

Don't let pure technical aspects slow you down. Eurogentec has optimised everything to let you focus on your research goals and reach them faster.

The coupling principle involves the biotinylation of your antibody followed by linking it to a streptavidin-coated bead. Example of the basic downstream process is described below.

## Technical features

- We use 1  $\mu\text{m}$  beads, with the most narrow size distribution
- High and constant magnetic content
- Perfect compromise between size, magnetic content and sedimentation property
- Optimized binding surface
- Allows easy and gentle handling of proteins

The coupling service is available for any polyclonal or monoclonal custom antibody made by Eurogentec. To learn more about custom antibody production at Eurogentec, and especially our unique 28-day Speedy immunisation, please visit our website [www.eurogentec.com](http://www.eurogentec.com)

We also offer the service for any antibody you provide us. Please contact us to learn about conditions applying to sample format and shipment.



## Magnetic beads coupling services

### Description

Coupling of your antibody to magnetic bead includes:

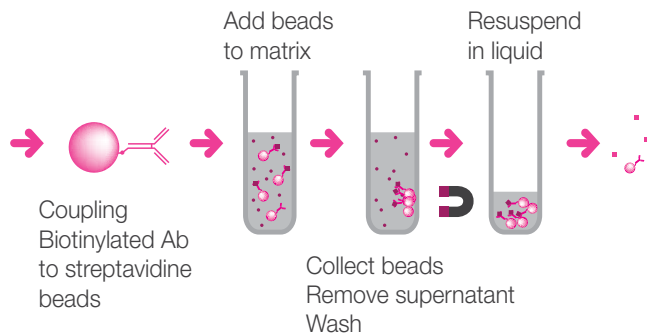
- ⇒ Sample preparation
- ⇒ Antibody biotinylation
- ⇒ Coupling of 60  $\mu\text{g}$  of biotinylated Ab to  $3-8 \cdot 10^8$  1  $\mu\text{m}$  magnetic beads
- ⇒ QC and report

### Reference

AS-MMCO-0160

Custom antibody made by Eurogentec

Coupling to biotin  
Your antibody



- > SDS PAGE analysis
- > Preparative isolation
- > Bead ELISA
- > Immunoprecipitation
- > Protein Pull-down

