

Antibodies

For Life Science Research

- > **Custom monoclonal** antibodies
- > **Custom polyclonal** antibodies
- > Custom Ab **fragments**



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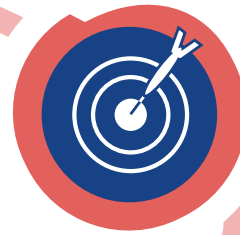
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IN CONSTANT INNOVATION

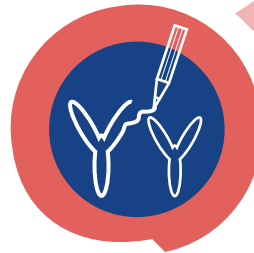
To follow the latest trends in the field of antibodies and to be at the forefront of technological innovation, we continuously improve our production facilities and services. This enables us to offer you the widest choice among cutting-edge antibodies and best programme options.



Stable and **trusted** antibody services since 1996



More than **1500 programmes** every year



From ready-to-use **catalogue** antibodies to fully **customised** production programmes



From **research** antibodies to **GMP** production of antibody fragments



Highly skilled **project managers** dedicated to your project

Eurogentec Antibodies

Custom

Catalogue

Polyclonals

Monoclonals

Therapeutics

Additional
services

Primary
and secondary

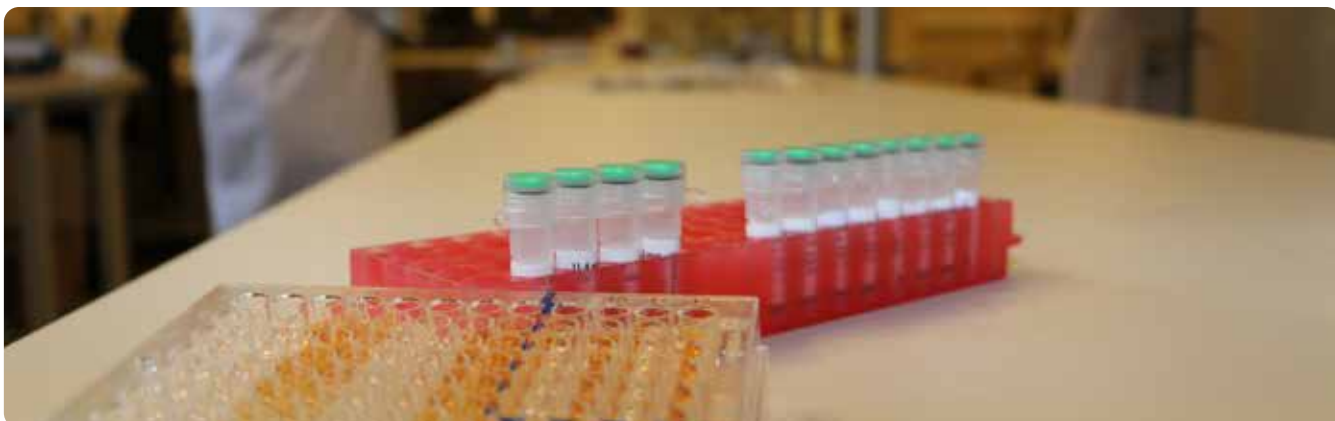
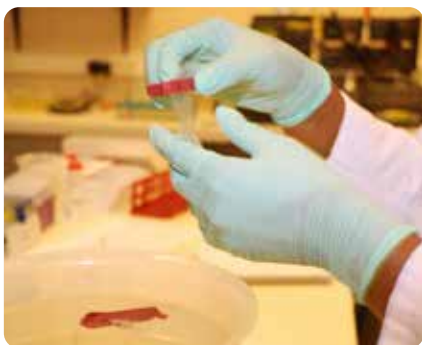
➤ p7	Custom antibodies
➤ p8	Host selection
➤ p9	Polyclonal antibodies
➤ p14	Monoclonal antibodies
➤ p16	Therapeutics
➤ p18	Additional services

Annexes

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YOUR ANTIBODY SOLUTION PROVIDER

In addition to our high-quality catalogue antibodies, we offer the most flexible and comprehensive services for the production of custom antibodies. Taking advantage of our state-of-the-art expertise and technologies, we can design the antibody programme that best suits your research.



Eurogentec Know-how

Scientific advise and support

- Eurogentec has long been recognised for its quality support regarding catalogue antibodies. This first line support service is available by phone, e-mail or live chat at any stage of your project.
- For any custom programme our project managers will guide and support you all along your specific antibody development.

Antigen

- Eurogentec's antibody team offers a high level expertise in the design and synthesis of immunogenic peptides as well as in the production of recombinant proteins or DNA.
- Pathogenic antigens are handled with the appropriate precautionary measures in the biosafety level 2 zone of Eurogentec's facilities.

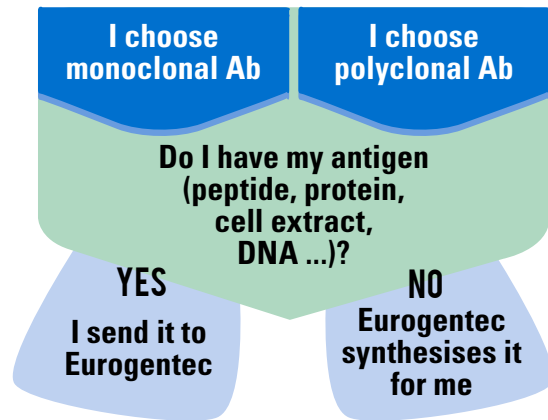
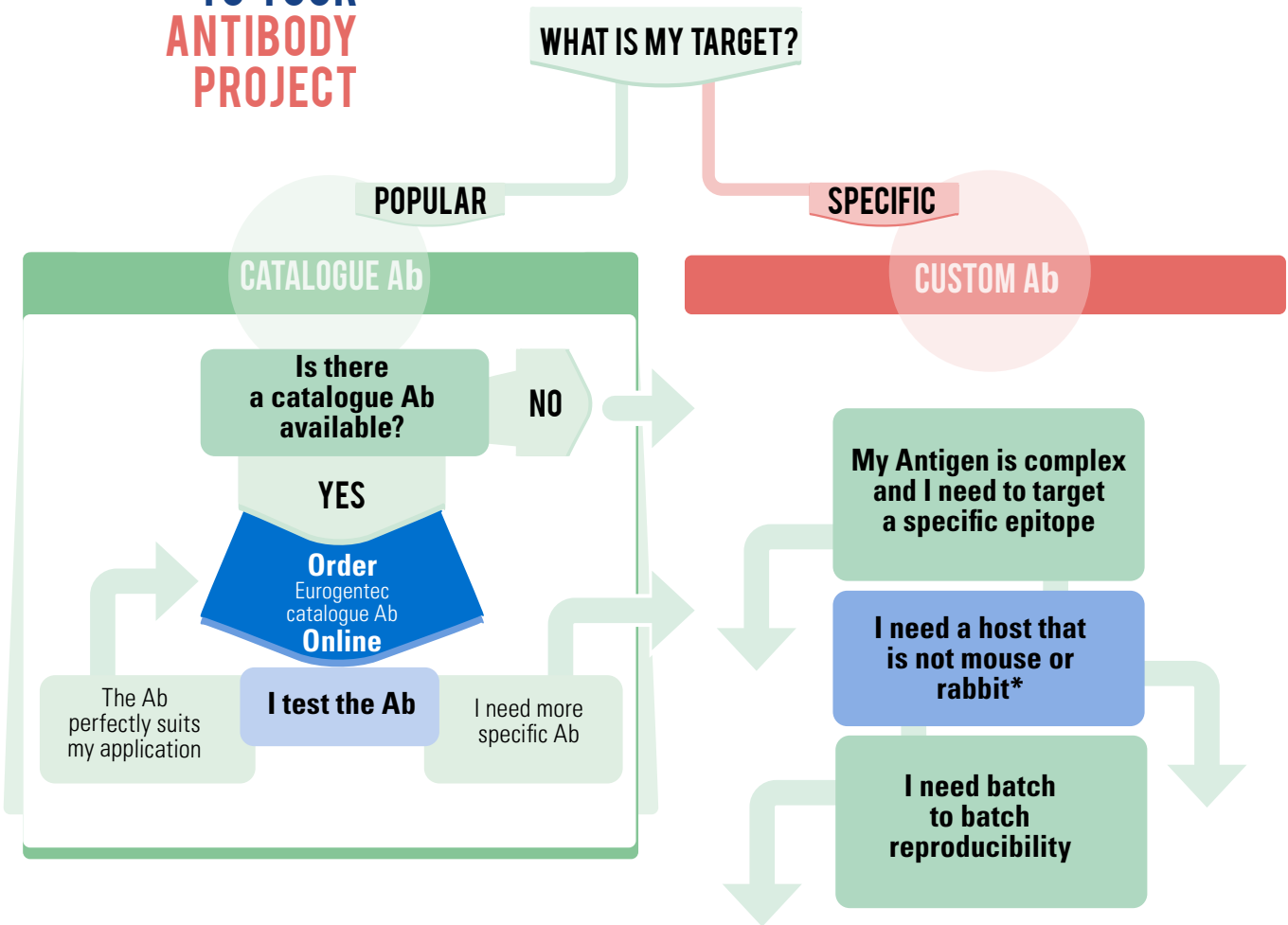
Immunisation

- Our proprietary Speedy 28-Day polyclonal programme reduces the immunisation time to 28 days. It uses a combination of non-Freund's adjuvants that stimulates the host's immune response and generates a high IgG/IgM ratio.

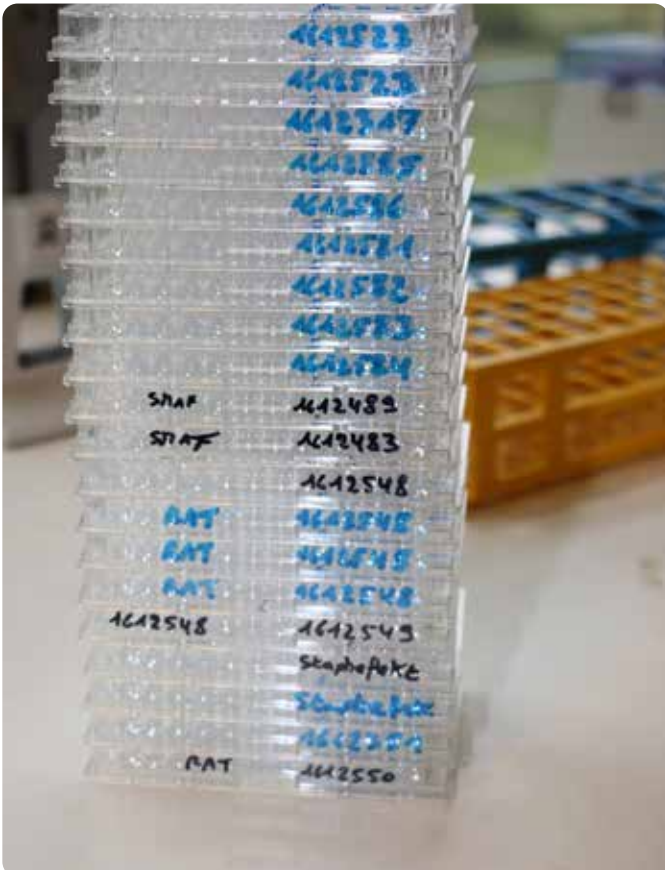
Antibody

- Eurogentec has distributed ready-to-use catalogue Abs for many years. Based on this long-standing expertise, we now offer a new range of top-quality primary antibodies.
- Eurogentec is a pioneer in the *in vitro* production of monoclonal antibodies. Since 2004, we have continuously improved and optimised the culture conditions to get the most out of your hybridomas.
- Eurogentec uses the largest animal facility in EU allowing us to manage numerous, -small to very large- antibody projects.
- For therapeutics applications, we provide custom V_HH and mAb fragments, which can be produced under ISO 13485 and GMP standards.

ROADMAP TO YOUR ANTIBODY PROJECT

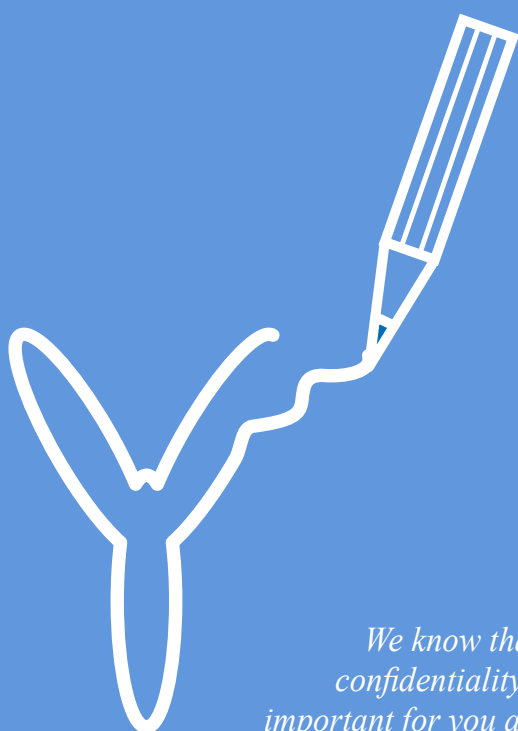


* Both monoclonals and polyclonals can be raised in mice and rabbits (please note that hybridoma development in Rabbits is patent-protected).



<http://bit.ly/white-paper-ab>

Custom antibodies



Whether you are a research laboratory, a university centre or a pharmaceutical company, Eurogentec offers you a range of flexible antibody services tailored to your specific needs.

Immunisation in diverse hosts can be performed with several antigens such as peptides, proteins, cell extracts or DNA. These antigens can be provided by you or produced by Eurogentec. We are experts in the generation of polyclonal (pAb) and monoclonal (mAb) antibodies for simple projects to large and high-demanding projects.

We offer a continuum of production capabilities including manufacturing for therapeutic applications. We can produce your recombinant Ab fragment (including Fab, scFv and nanobody) in our FDA inspected GMP Biomanufacturing facility for use in human clinical trials. ■

We know that confidentiality is important for you and we handle your data and projects with utmost care and respect. All hybridoma, sera and results obtained belong to the customer and will remain as a property of the customer.*

Eurogentec guarantees that it will not claim any rights on the hybridoma or the antibodies. If desired, a Non-disclosure Agreement concerning our production and the customer's purpose can be executed.

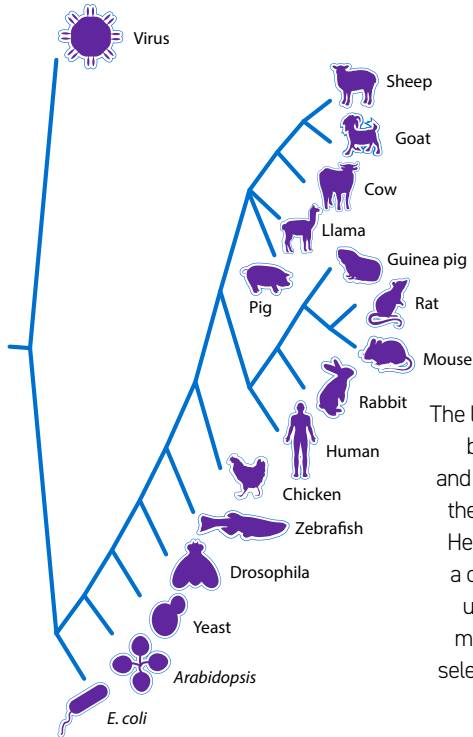


*To be discussed in case of DNA immunisation

THE BEST HOST FOR YOUR PROJECT

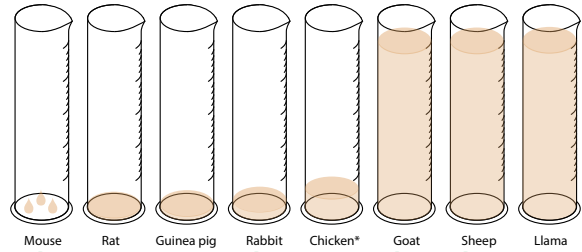
KEY FACTORS

Antigen homology



The lower the homology between the antigen and the host, the higher the immune response. Hence, the selection of a chicken host may be useful if a conserved mammalian protein is selected as the antigen.

Ab quantity



Avoid immunisation of several small animals in parallel

Suitable for most of the projects

Useful to compare immunogenicity of various antigens

* In average, 8 to 10 egg yolks will be harvested per chicken immunisation (depending on the laying). 4 eggs contain as much antibody as the serum from one rabbit.

Applications

Detection

If a secondary Ab is to be used in the final application, one may favour one host over another based on the availability of this secondary Ab.



Less frequent



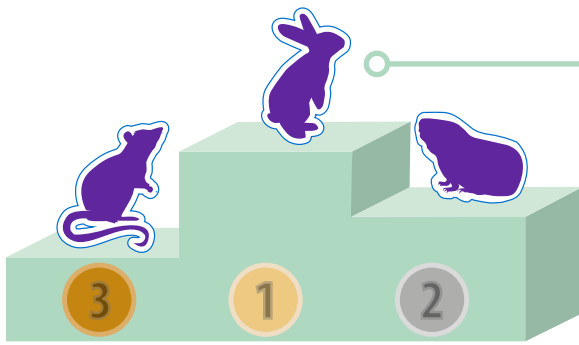
Low reproductibility

Therapeutics



We produce polyclonal and monoclonal Llama antibodies and collaborate with QVQ to provide V_HH fragments. Their small size makes them of high value for therapeutics interest.

Usage



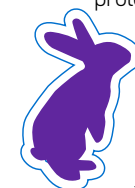
Rabbit is the most widely used host for pAb production because of its easy maintenance and efficient immune system. Moreover, the quantity of serum collected is suitable for most of the projects.

Rats and guinea pigs are recommended for small quantities of high quality polyclonal antibodies at an affordable price.

mAb



Mice are ideal for mAb production see p14



Rabbits mAbs are patent-protected; Eurogentec can assist you by immunising the animal, checking the immune response and isolating the B-cells from the spleen.



Custom polyclonal antibodies

Eurogentec provides a wide range of efficient custom polyclonal programmes extending from classical to the fastest proprietary programme, Speedy 28-Day.

You can also benefit from our expertise in the production of your antigen either peptides or proteins. ■

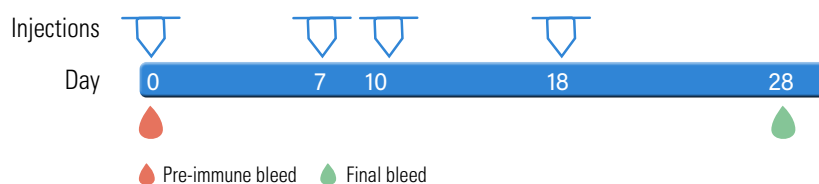
⇒ SPEEDY 28-DAY

Immunisation lasts 28 days

Recommended for

- > A strong and quick response
- > An anti-PTM programme
- > A quick access to a small quantity of purified and ready to use pAb (with the 'Speedy Mini' programme)

IMMUNISATION SCHEDULE



All bleeds are sent to the customer

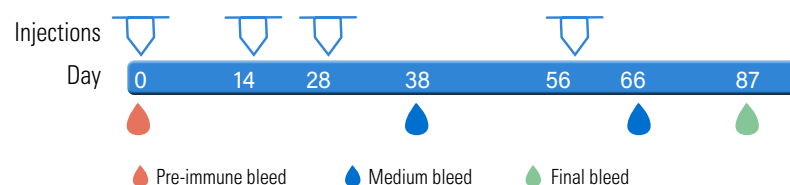
⇒ CLASSICAL

Immunisation lasts from 65 to 90 days depending on the host and the immune response.

Recommended for

- > Repeating a pre-existing protocol already conducted with a classical programme
- > Immunising a host where the Speedy 28-Day programme is not possible or not guaranteed (chicken, mouse, large animals,...)
- > An antigen of poor immunogenicity that may require prolongation of the programme

IMMUNISATION SCHEDULE*



All bleeds are sent to the customer

* This example is subject to variations (injections and intermediate bleeds) depending on the programme selected.

GOOD TO KNOW

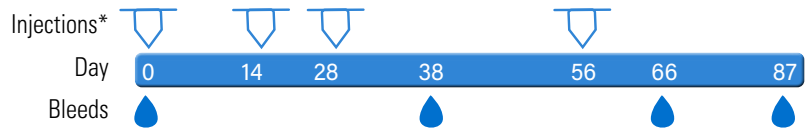
** Eurogentec offers **recombinant protein** production on request: proteomics.services@eurogentec.com

Additional services including antibody coupling, labelling and additional purifications are available. See p.19 for more information ■

HOW TO START
SEE PAGE 26

INTERESTED IN

LARGE PROJECT SEE PAGE 27
LLAMA IMMUNISATION SEE PAGE 17



Classical programmes

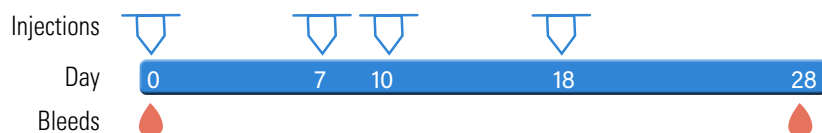
		Anti-antigen		Anti-peptide		Double X		Anti-PTM**			
		You provide the antigen^a (peptide, protein, complex sample)		Eurogentec designs and synthesizes one peptide		Eurogentec designs and synthesizes two peptides to increase the chances of success		Eurogentec designs and synthesizes two peptides: one modified with the PTM and one unmodified			
Peptide	Length	✗		10 - 16 aa	10 - 16 aa each	10 - 13 aa each					
	Quantity			15-25 mg	15-25 mg each	15-20 mg each					
	Purity			>70 %	>70 %	>70 %					
	Coupling to a carrier (KLH, BSA, OVA, THY or MAP carrier)	⊕		5 mg	5 mg each	5 mg of modified peptide					
Immunisation	Host ^b	Rabbit	Nbr	Cat#	Nbr	Cat#	Nbr	Cat#	Nbr	Cat#	
			2	AS-PNOR-3MORAB	2	AS-PCAP-RABBIT	2	AS-DOUB-LX	2	Phospho	AS-PSPE-CIFIC
										Acetyl Lys	AS-PTMA-LYSACET
										DiAcetyl Ser	AS-PTMA-SERACET2
										Oxidized Cys	AS-PTMA-OXCYS
										MonoMethyl Arg	AS-PTMA-ARGME1
										DiMethyl Arg (sym)	AS-PTMA-ARGME2SYM
										DiMethyl Arg (asym)	AS-PTMA-ARGME2ASY
										MonoMethyl Lys	AS-PTMA-LYSME1
										DiMethyl Lys	AS-PTMA-LYSME2
								TriMethyl Lys	AS-PTMA-LYSME3		
		Rat	2	AS-PNOR-3MORAT	2	AS-PCAP-RAT	2	AS-DOUB-LXRAT	6	Phospho	AS-PSPE-CIFICRAT
		Guinea pig	2	AS-PNOR-3MOGPG	2	AS-PCAP-GUIPG	2	AS-DOUB-LXGUI	6	Phospho	AS-PSPE-CIFICGP
	Goat	1	AS-PNOR-3MOGOAT	1	AS-PCAP-GOAT	1	AS-DOUB-LXGOAT	✗			
	Mouse	3	on request	3	AS-PCAP-MOUSE	3	on request	✗			
	Chicken	2	AS-PNOR-3MOHEN	2	AS-PCAP-HEN	2	AS-DOUB-LXHEN	2	Acetyl Lys	AS-PTMA-LYSACETHEN	
	Large animal (pig, sheep, cow,...)	1	on request	1	on request	1	on request	✗			
									More modification on request		
	ELISA		✗		⊕		⊕			⊕	
Antibody Processing	Affinity Purification		⊕		⊕		⊕		Double purification of 50 mL serum		
	QC validation		⊕		⊕		⊕		ELISA and SDS-PAGE		
	Delivery		Serum Purified antibody (Optional)		Serum Purified antibody (Optional) Remaining peptide		Serum Purified antibody (Optional) Remaining peptide		Purified antibody Remaining serum Remaining peptides		

✗ = not applicable ⊕ = Optional

- a. See the section "How to provide my antigen" on p.25 for more details
- b. Other hosts are available on request
- c. The guarantee only applies for peptides when approved by Eurogentec
- d. The guarantee does not apply for Goat immunisation

* This example is subject to variations (injections and intermediate bleeds) depending on the programme selected.

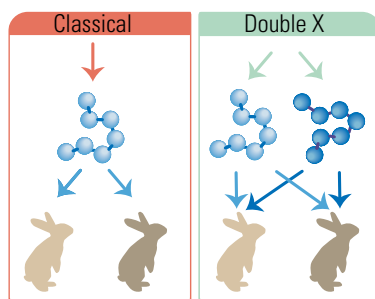
**Post Translational Modification



Speedy 28-day programmes

Anti-antigen		Speedy-Mini		Anti-peptide		Double X		Anti-PTM**	
You provide the antigen^a (peptide, protein, complex sample)		Eurogentec designs and synthesises one peptide		"Eurogentec designs and synthesises one peptide "		Eurogentec designs and synthesises two peptides to increase the chances of success		"Eurogentec designs and synthesises two peptides: one modified with the PTM and one unmodified"	
✗	10 - 16 aa	10 - 16 aa	10 - 16 aa	10 - 16 aa each	10 - 13 aa each				
	8-10 mg	15-25 mg	15-25 mg each	15-20 mg each					
	> 70 %	> 70 %	> 70 %	> 70 %					
+	2-3 mg to KLH	5 mg	5 mg of each	5 mg of mofied peptide					
Nbr	Cat#	Nbr	Cat#	Nbr	Cat#	Nbr	Cat#	Nbr	Cat#
2	AS-SUPR-ANTIGEN	1	AS-SMAF-SINGLE	2	AS-SUPR-SINGLE	2	AS-SUPR-DX	2	Phospho AS-SUPR-PSPEC
									Acetyl Lys AS-SUPR-LYSACET
									Citruline AS-SUPR-CITSPEC
									Oxidized Cys AS-SUPR-OXCYS
									MonoMethyl Arg AS-SUPR-ARGME1
									DiMethyl Arg (sym) AS-SUPR-ARGME2SYM
									DiMethyl Arg (asym) AS-SUPR-ARGME2ASY
									MonoMethyl Lys AS-SUPR-LYSME1
									DiMethyl Lys AS-SUPR-LYSME2
2	AS-SUPR-RATANTIGEN	✗		2	AS-SUPR-RATSINGLE	2	AS-SUPR-RATDX	6	on request
2	AS-SUPR-GPANTIGEN	✗		2	AS-SUPR-GPSINGLE	2	AS-SUPR-GPDX	6	Phospho AS-SUPR-PSPECGP
1	AS-SUPR-GOATAG	✗		1	AS-SUPR-GOATSING	1	AS-SUPR-GOATDX	✗	
✗		✗		✗		✗		✗	
✗		✗		✗		✗		✗	
✗		✗		✗		✗		✗	
									More modification on request
✗			Specific response at 1/20000 guarantee ^c No Result, No Serum, No Invoice ^d		Specific response at 1/20000 guarantee ^c No Result, No Serum, No Invoiced		Specific response at 1/20000 guarantee ^c (at least for one of the 2 peptides) ^d		+
+			Purification of 10 mL Serum	+		+			Double purification of 50 mL serum
+			ELISA		ELISA		ELISA		ELISA and SDS-PAGE
	Serum Purified antibody (Optional)		Serum Purified antibody		Serum Purified antibody (Optional) Remaining peptide		Serum Purified antibody (Optional) Remaining peptides		Purified antibody Remaining serum Remaining peptides

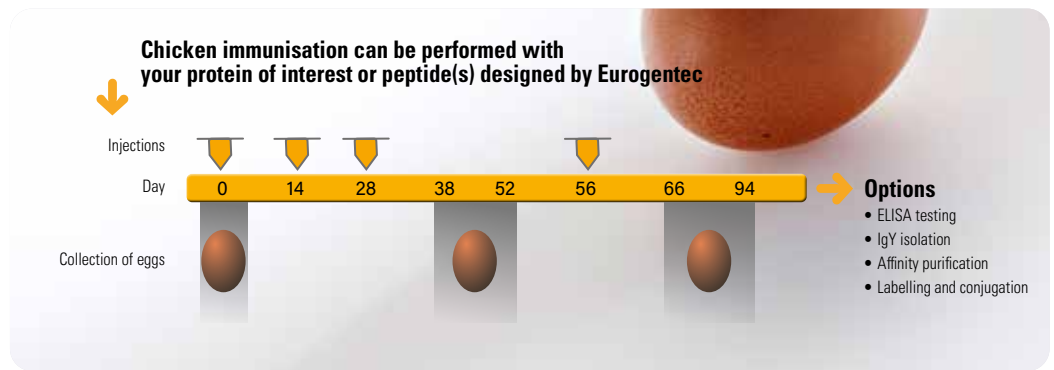
Double X programmes



Double X programmes increase the chances of success by using two peptides corresponding to distinct regions of the targeted protein. Each host will receive a combination of the two peptides.

Chicken immunisation

Using a non-mammalian host for immunisation allows considering **even highly conserved** mammalian proteins as targets. Moreover, hens produce antibodies in their eggs, avoiding the animal final bleed. ■



Advantages of choosing a chicken immunisation programme

- Using chicken IgY often leads to **less background** in your application.
- It allows targeting **mammalian proteins** that are highly conserved among classical hosts.
- It produces **high quantity of IgY antibodies**: 4 eggs contain as much antibody as the serum from one rabbit (200 mg total IgY containing 2-10% antigen-specific IgY).
- You will receive 10 to 20 eggs yolks harvested within 90 days (one egg yolk pool by hen)*
- We perform IgY purification from egg yolk.

Post Translational Modifications

(PTMs)

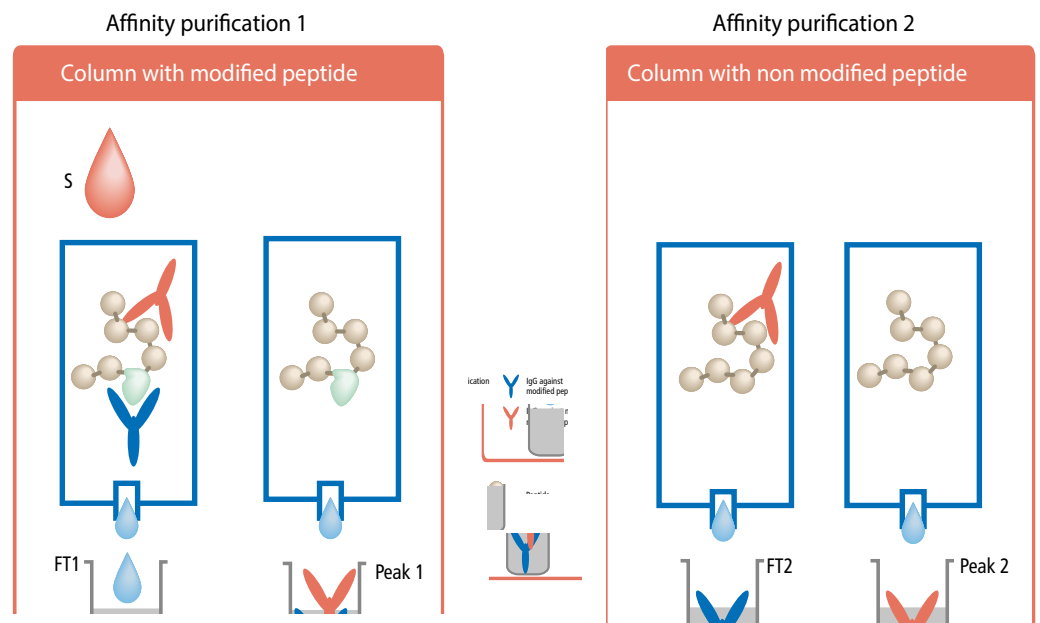
Thanks to our strong expertise in producing antibodies against PTMs, we can target classical modifications as well as more complex ones. The Speedy 28-Day programme is particularly adapted for the generation of antibodies against PTM due to the high frequency of the injections. ■

Phospho-
Acetyl-
DiAcetyl-
MonoMethyl-
DiMethyl-
TriMethyl-
Oxidised-
Citruline-

And more on request

BE AWARE OF

The purification of antibodies against PTM requires a double purification step. As a result, the titer of the antibody is reduced but its specificity against the modification is optimal. ■



PTM programme step by step

- Design and synthesis of 2 peptides (1 non-PTM + 1 harbouring the PTM)
- Coupling of the PTM peptide to a carrier
- Immunisation of the hosts using the PTM peptide following the Speedy 28-day or a classical 87-day protocol
- Analysis of the immune response by ELISA and if applicable, selection of the best responding host
- **Double affinity purification of the pAb using the PTM and subsequently the non-PTM peptide (see figure above)**
- ELISA testing of the purified pAb against the non-PTM and PTM peptides
- Shipping

NOTE

* In average, 8 to 10 egg yolks will be harvested per chicken immunisation (depending on the laying).



Options

for your polyclonal programme

1. Screening

Pre-immune sera testing

Screening the pre-immune serum can be performed on several animals to select the one with the lowest background in your application. For this purpose, you will receive 5, 10 or 20 pre-immune sera samples from different animals to allow you selecting the one(s) best suited for your application.

2. ELISA

Immune response monitoring

ELISA is an excellent option to follow the evolution of an immune response during an immunisation.

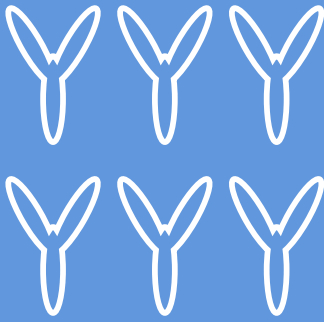
The ELISA tests are carried out in one 96-well plate per animal.

We test in parallel, per ELISA, dilutions from:

- Pre-immune sera
- Small bleed
- Large bleed

Against

- The free peptide
- The carrier protein
- Positive and negative controls.



Custom monoclonal antibodies

Eurogentec develops hybridomas in mouse and produces mg to g scale mAb exclusively *in vitro*.

We offer cell banking facilities for a backup of your hybridomas whether you are a research laboratory or a pharmaceutical company. ■

HYBRIDOMA GENERATION

Eurogentec develops murine hybridomas in 16-19 weeks following a stepwise approach with possible testing sample phases. A reporting is sent after each phase and can be discussed with the customer for potential adaptation. If no positive hybridoma is obtained after phase 3, only phase 1 will be charged. ■

PROJECT DEVELOPMENT

Prior to project initiation, you can benefit from our personalised project proposals and expertise.

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**

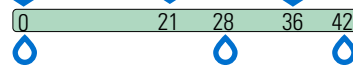
STARTING MATERIAL

You send us:

- * Your purified **protein**
- * Or your **peptide sequence**
- * Or your protein's **accession number**
- * Or other **antigen**

PHASE 1: IMMUNISATION

Immunisation of **4 mice** (6 weeks)

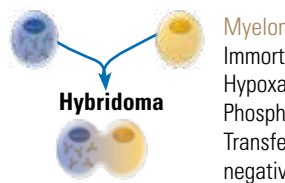


You receive:
ELISA report against the supplied antigen and serum sample

PHASE 2 : FUSION/HYBRIDOMA PRODUCTION

2 - 3 weeks

Mouse lymphocytes
Specific Antibody
Secreting Cells
Ig positive



Hybridoma

Spleen cell + Myeloma cell Sp2 OAg 14

You receive: Fusion report

CUSTOMER REQUEST



NO

ELISA titer

YES

NO

Hybridoma

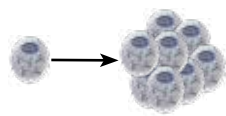
IN VITRO PRODUCTION

STARTING MATERIAL



Your **hybridoma**
(coming from you
or from
Eurogentec)

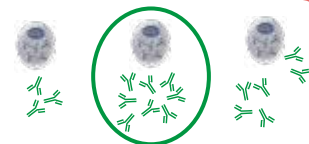
CLONE EXPANSION



Production
> 200 mg

YES

TEST PHASE



Assessment of the clone productivity

NO

In bioreactors

Ad



In roller bottles

BENEFITS

- **Efficiency:** highly reproducible production of high quality mAbs
- **Flexibility:** a large and comprehensive panel of services
- **Attractivity:** a production batch using a normal hybridoma producer usually yields enough antibody material for an entire project!
- **Rapidity:** the production lasts only one month

In vitro production

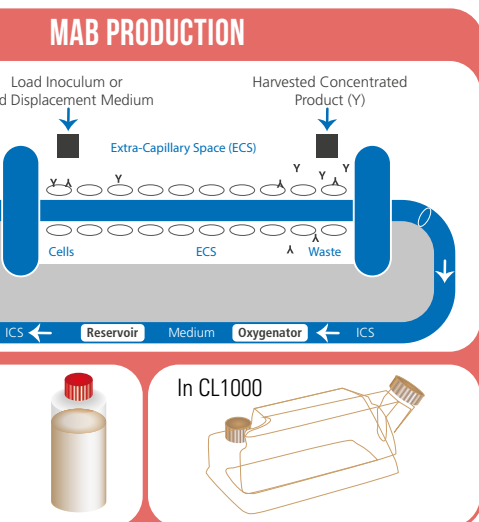
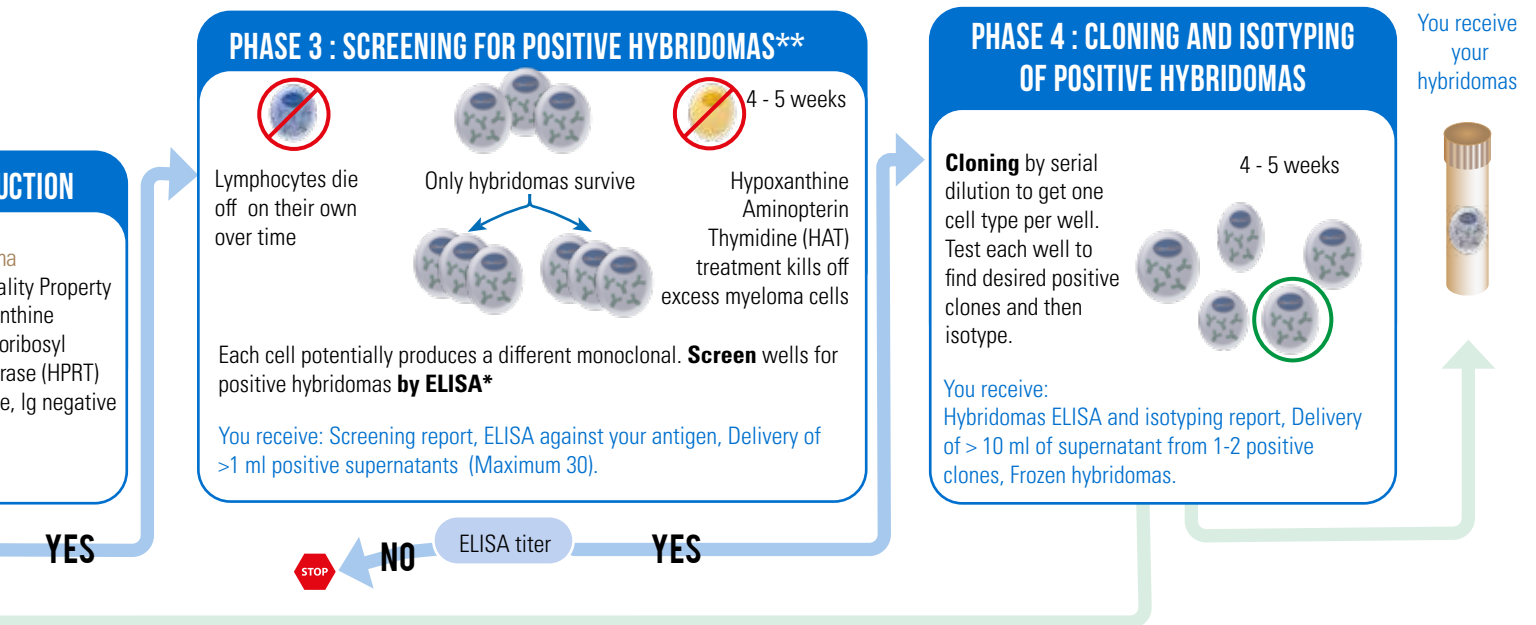
The European legislation 2010-63-UE highly encourages producing mAbs in vitro, and more and more countries now prohibit the mAb production in ascites, a method associated with animal pain and distress. Prior to this recommendation, Eurogentec already switched to the in vitro production of mAbs and therefore developed a deep expertise as well as highly efficient production protocols. We perfectly control the production of mAbs from flasks to bioreactors (>1000 L). ■

HOW TO START

SEE PAGE 26

Do you need rabbit monoclonals?

Rabbits mAbs are patent-protected. Eurogentec can assist you by immunising the animals and checking the immune response, then removing the spleen and isolating the B-cells.



GOOD TO KNOW
 Eurogentec uses hollow-fibre technology based bioreactors. This system gets the most out of hybridomas and produces large amounts of mAbs. ■

PURIFICATION
 You choose the purification:
 * Protein A
 * Protein G
 * Affinity
 * No purification

AB PROCESSING
 Various antibody **options** (labelling, fragmentation, sequencing, choice of different buffers, mAb concentration, lyophilisation,)
 You receive your antibody

* Due to the instability of hybridomas during the screening phase, the screening step (phase 3) is critical. By default, hybridomas are selected based on the culture optical density (OD). If you need any specific screening method, we invite you to discuss prior to phase 1 with our experts for the smooth progress of the project. The test phase will be adapted according to your screening method.

** Hybridomas are considered as positive when they generate a positive signal in ELISA against the antigen, based on analysis criteria defined by Eurogentec.

Therapeutics

For therapeutic uses, very pure and efficient molecules are required, while being small enough to be stable *in vivo*. Antibody fragments are good candidates for such therapeutic applications. For this purpose, we have developed proprietary plasmids to produce GMP antibody fragments. In parallel, together we immunise llamas and generate custom llama V_HH. ■



➤ ANTIBODY FRAGMENTS

mAb fragments

Eurogentec has renowned expertise in the capacity to produce large amounts of recombinant proteins including Ab fragments in patent free strains of *Pichia pastoris*. Multicopy clones are isolated using Eurogentec's proprietary plasmids (high yield). To start producing your antibody fragments, you have to provide the sequence of your choice.

All antibody fragments can be produced:

- Fabs
- Nanobodies
- scFv
- And more

CONTACT

info.biologics@eurogentec.com

GOOD TO KNOW

Eurogentec is an expert in protein production in various strains. If you dispose of a specific strain expressing your Ab fragment, contact us for a feasibility study.

Since 1996, we have established trusted collaborations with many Big Pharma's and biotechnology companies around the world to develop and produce **clinical trial materials**. ■

BENEFITS

GMP biomanufacturing of Ab fragments

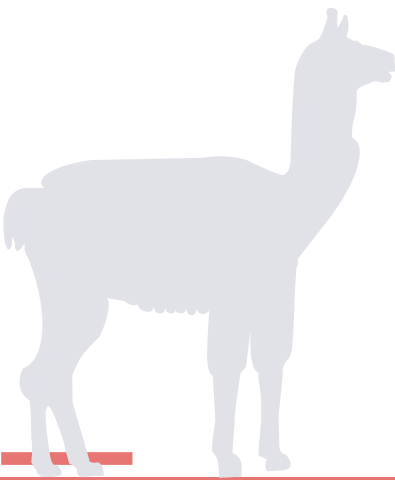
From small to large scale production

Full V_HH service from llama immunisation to V_HH production



Camelid antibodies and monoclonal V_HH production

Camelids produce single-chain antibodies in addition to conventional ones. The antigen binding domains of these antibodies, called V_HH, are the smallest naturally occurring antibody fragments that recognise the antigens. We accompany you from llama immunisation to V_HH production including lymphocyte isolation and mRNA extraction. Eurogentec can label your V_HH fragments on request. ■



BENEFITS

Better targeting

Due to their small size (~ 15kDa), V_HH can also bind epitopes that are hidden, hence targeting precision is higher compared to normal antibodies.

Easier production

The small size of V_HH makes them relatively easy to produce in lower eukaryotes up to very high amounts and purities.

Unique properties

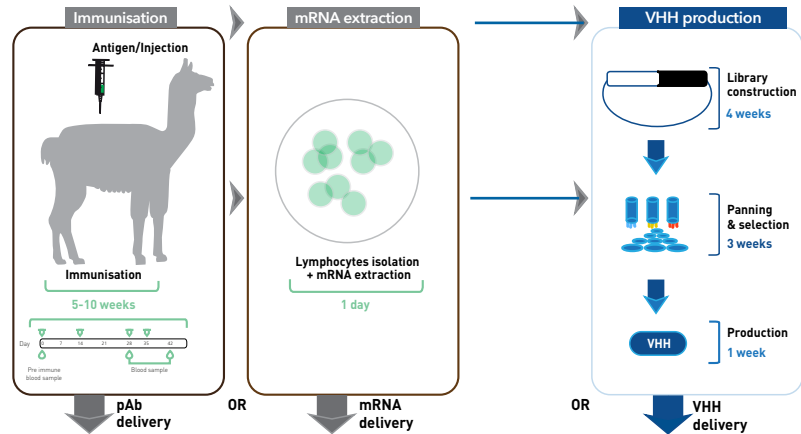
V_HH have a high tissue penetration and are cleared from circulation rapidly. Some V_HH can even cross the blood-brain barrier.

Higher stability

V_HH are stable under extreme pH, temperature and against proteases; hence, they keep their native folding and epitope binding capacity under very diverse experimental conditions.

We generate llama polyclonal antibodies targeting multiple antigens, and offer a wide range of additional services.

We generate V_HH phage display libraries and perform selection and screening of high affinity monoclonal V_HH.



Immunisation

- > Host: Llama or Alpaca
- > Programme: customer dependent; 5 to 10 weeks
- > Antigen: from the customer or produced at Eurogentec. Protein amount required per immunisation: 0.5 mg/llama [2 llamas are scheduled for immunisation]
- > Standard immunisation schedule: Pre-immune bleed (day 0); 4 injections (day 0, 14, 28 and 35), 2 bleeds (day 28 and 42).
- > Optional: Testing of immune response. ELISA testing against the antigen is generally performed. This step may require an additional amount of antigen.

> **Deliverables:** the programme can be stopped here and you receive pAb (crude serum or purified pAb).

RNA extraction

The lymphocytes can be isolated from a blood sample (+/- 250mL) followed by RNA extraction. Please note that lymphocytes isolation and RNA extraction must be performed very rapidly after the blood sampling to avoid RNA degradation. RNA is checked for quality prior to sending.

> **Deliverables:** the programme can be stopped here and you receive RNA.

Monoclonal V_HH selection

The service of V_HH generation includes one or several of the following steps:

> Library construction

> **Deliverables:** two libraries with size >10⁷ different clones and >90% insert.

> Selection and screening for high affinity V_HH clones + sequence determination.

> **Deliverables:** the sequences of at least 6 binders from 2 families.

> Production, V_HH single domain antibody production and purification.

> **Deliverables:** 0.5 mg protein of the lead clones produced in *E. coli* and purified (>90%).

ADDITIONAL SERVICES

1. Large scale production in yeast for animal experiments or crystallography up to GMP production for microdosing studies in human.
2. Optimisation of V_HH sequence for production, physical and "in product" stability and various types of clinical use.
3. Formatting of the V_HH in bivalent, bispecific or custom made formats.
4. V_HH can be custom labelled.



Additional Services

ELISA testing

Eurogentec can assay your antibody in various applications including ELISA and western-blot. ■



Purification

We can perform purification of total IgG (IgY in chicken) or affinity purification using the antigen. ■



Labelling

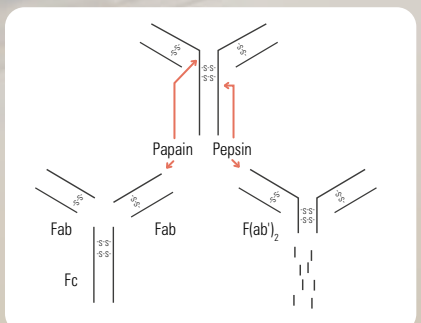
To avoid using a labelled secondary antibody, your specific primary antibody can be labelled with the molecule of your choice. We offer a large panel of dyes and labels (refer to our Protein iDentification brochure).

- **Fluorophores** are ideal for flow cytometry and fluorescent microscopy. They include AnaSpec's proprietary high-quality fluorophores HiLyte™ Fluor and CyLyte Fluor.
- **Alkaline phosphatase (AP)** allows the detection of an antibody with colorimetric substrates.
- **Horse Radish Peroxydase (HRP)** is detectable by colorimetry and by chemiluminescence that offers a better sensitivity than colorimetry.
- **Biotin** reacts extremely specifically with Streptavidin substrates. ■

Fragmentation

It has been shown that many cells have receptors for the Fc fragment of antibodies leading to unspecific background. To avoid such a background signal, Eurogentec selectively cleaves your antibodies into fragments and provides you with Fab or F(ab')₂. ■

Please note that chicken IgY do not bind to the Fc receptors expressed on the surface of mammalian cells, hence generating less background (see p.12)

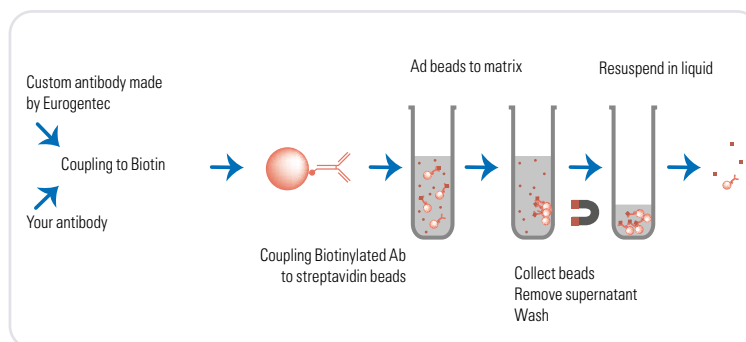


ALSO AVAILABLE

- Secondary antibodies
- Protein A coupled to fluorescent dyes and biotin, see p. 23 ■

Coupling to magnetic beads

Eurogentec has joined forces with a state of the art beads manufacturer to couple your antibody to magnetic beads. To ensure the success and convenience of your experiments, we selected the best beads regarding size distribution, magnetic content consistency, binding surface, sedimentation property, easiness of protein handling and size. ■



APPLICATIONS

- Preparative purification
- Bead-based ELISA
- SDS PAGE analysis
- Immunoprecipitation
- Protein Pull-down

Epitope mapping

To determine the protein epitope specifically recognised by an mAb, Eurogentec can design and synthesise a peptide library which spans the sequence of the target protein, and screen this library in a 96-well format to identify the peptide region which harbours the mAb epitope. ■

A. Peptide design

KNCSHIQPWETDCLSC LPERQDEYDPKGPK...

- P1. KNCSHIQPWETDC
P2. QPWETDCLSC LPE
P3. CLSC LPERQDEYD
P4. ERQDEYDPKGPK

OPTION

Synthesised peptides (≥ 10 aa) can be biotinylated to assure better and oriented fixation onto the support.

You provide

- The protein sequence
- The desired peptide length (13 mer peptide are suggested)
- The desired overlap (7 aa overlaps are suggested)

You receive

- Assistance with the peptide design
- The synthesised peptides (96 well formats), biotinylated on request

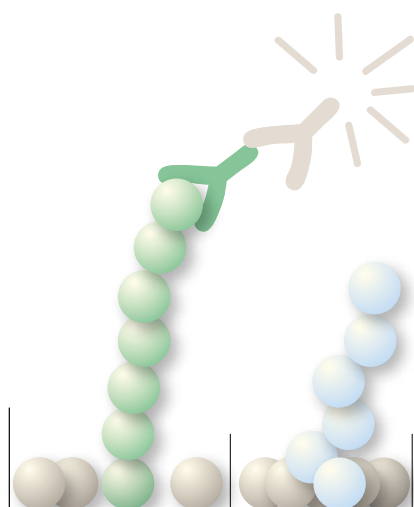
B. Library screening and epitope characterisation

You provide

- The Primary Antibody (purified; 1 mg/mL in PBS shipped on dry ice)
- A positive control (optional)
- Information about the species in which the primary Ab was raised

You receive

- ELISA Analysis Report
- The sequence of the Ab binding site(s)



*OPTION
Streptavidin precoated plate may be used to capture biotinylated peptides.

1. Plate Coating
2. Screening for the Primary Antibody epitope
3. Detection using a labelled Secondary Antibody

One plate is coated with 24 different designed peptides in triplicate*

Addition of your Primary Antibody (dilution 1:1000)

Labelled secondary antibody:

- Anti-rabbit
- Anti-rat
- Anti-mouse
- Anti-guinea pig
- Anti-chicken
- Anti-human or other on request

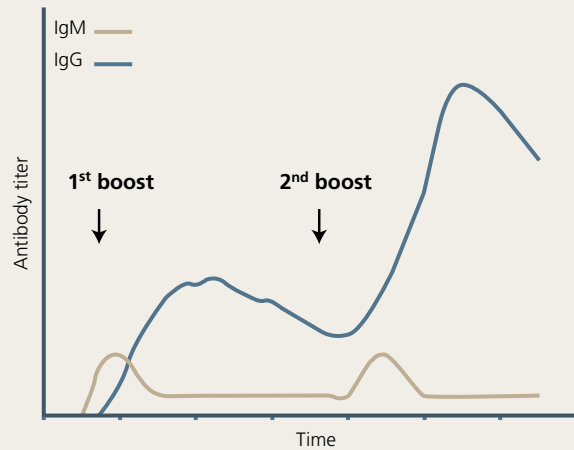
4. Epitope characterisation by ELISA

Selected positive peptides (triplicate) are incubated with various antibody dilutions (1:1000, 1:3000 and 1:9000)

ANNEXES

HOW TO UNDERSTAND Ab TITER EVOLUTION

IgMs are more difficult to label and detect than IgGs because of their pentameric structure. To obtain the optimal (highest) IgG/IgM ratio, Eurogentec's immunisation programmes are optimised concerning the number and frequency of the boosts. Special attention was paid to this ratio when developing the Speedy 28-day programme, to ensure a minimal amount of IgMs in the final bleed. IgM and IgG antibody titer as a function of time may be represented in the general scheme beside.



DISCOVER BLEED VOLUME

The immune response depends on the antigen but also on the host. Large animals provide the advantage of allowing larger bleeds but their immune response is generally lower than small animals. The following table indicates the expected bleed volume in various hosts.

Bleed volumes					
	Pre-immune	Small Bleed	Large bleed	Final bleed	Comment
Mouse	40-70 µL	40-70 µL	40-70 µL	300-500 µL	Good to test antigenicity
Guinea pig	1 mL	1 mL	2-3 mL	10-15 mL	For small serum volumes
Rat	2 mL	2 mL	2 mL	5 mL	For small serum volumes
Chicken	1 egg	± 8-10 eggs*	± 8-10 eggs*	± 8-10 eggs*	For mammalian antigens and large quantities of Antibodies, 4 eggs = 1 rabbit final bleed
Rabbit	2 mL	2 mL	20-25 mL	50-70 mL	For most applications
Goat	2 mL	2 mL	250 mL	1000 mL	For large batch volumes
Sheep	2 mL	2 mL	250 mL	1000 mL	For large batch volumes
Llama	2 mL	2 mL	250 mL	1000 mL	Single chain antibodies and for large batch volumes

Typical bleed volumes in the most common hosts

* On average, depending on the laying

PROTEIN A AND G AFFINITY

Depending on the host, protein A and G do not have the same affinity for immunoglobulins. The following table gives an overview of the relative affinities of IgG (and IgY) from different species for protein A and protein G.

Origin of Immunoglobulins	Protein A	Protein G
Mouse	+++	++++
Rat	-	+++
Guinea pig	++++	++
Rabbit	++++	+++
Goat	+/-	++
Sheep	-	++
Pig	+++	+++
Chicken (IgY)	-	-

Binding capacities of immunoglobulins from different species to protein A and G

Ship your antigen to Eurogentec

Antigens should be shipped along with a copy of the order form. Should you be shipping additional antigen for an on-going programme please indicate on the accompanying copy of the order form the antibody programme number that your antigen should be used for.

Shipping address:

EUROGENTEC
Immunisation Department,
LIEGE Science Park,
Rue du Bois Saint-Jean 5,
4102 SERAING, Belgium

ANTIGEN AMOUNT

Injection amounts per rabbits depend on the antigen weight:
100 µg per injection for
< 18 – 20 kDa proteins*;
200 µg per injection for
> 18 – 20 kDa proteins.

Injection amounts for other hosts are listed in the table beside.

Host	Min. antigen quantity / injection	
	Antigen MW < 18 kDa	Antigen MW > 18 kDa
Mouse	40 µg	15 µg
Guinea pig	50 µg	30 µg
Rat	50 µg	30 µg
Chicken	200 µg	100 µg
Rabbit	200 µg	100 µg
Goat	400 µg	200 µg
Sheep	400 µg	200 µg
Llama	400 µg	200 µg
Other	Contact us for recommendations	

Typical antigen amounts required for immunisation in the most common hosts.

AUTHORISED BUFFER COMPONENTS

Compound or formulations		
	Allowed	Not allowed
Water	Yes, keep the volume small (1 ml)	-
PBS	Yes, keep the volume small (1 ml)	-
Physiological buffer solutions	Yes, keep the volume small (1 ml)	-
Metal dyes/heavy metals	-	Risk of toxicity
Salts (KCl, NaCl, MgCl ₂)	< 1.0 M	> 1.0 M
SDS	< 2.0 %	> 2.0 %
Urea	< 6.0 M	> 8.0 M
Guanidinium HCl	-	Risk of toxicity
Digoxin/Digoxigenin	-	Risk of toxicity
Octylglucoside	< 1.0 %	> 1.0 %
Triton X-100/Tween-20	< 0.2 %	> 0.2 %
Glycerol	< 20 %	> 20 %
PMSF	-	Risk of toxicity
Pefabloc	< 0.1 mM	> 0.1 mM
Leupeptin/Pepstatin	< 1 µM	> 1 µM
DTT	< 3 M	> 3 M
Mercaptoethanol	-	Risk of toxicity
Imidazole	< 3 M	> 3 M
TFA	-	High risk of toxicity

Compounds and formulations that are acceptable for immunisation

ANTIGEN FORMAT

Lyophilised

You can send us your freeze-dried antigen at ambient temperature. If the antigen is poorly soluble in aqueous solution, we suggest that you avoid lyophilisation and that you send us your protein in solution on dry ice. The addition of adjuvant will help to dissolve even lipophilic proteins. If the antigen does not dissolve, a fine suspension of the antigen will be obtained by thorough mixing. Such fine suspensions can also be useful for antibody production because particles are efficiently phagocytosed.

SDS-PAGE**

For antibody productions with SDS-PAGE gel fragments, we advise our customers to cut out the band of interest and to aliquot it in separate tubes for each injection. The antigen tubes can be shipped at room temperature. The standard Coomassie and Coomassie-like staining procedure can be used since the Coomassie staining dyes do not interfere with the antibody evolution. However, silver stain is not allowed. The band should just be washed briefly but thoroughly in water to remove acetic acid and methanol residues, and then cut into injection pieces, and aliquoted wet into safe lock tubes to avoid drying. The gel must not be dried or lyophilised, because this would make the fragmentation before injection more difficult.

** Not available for Speedy programme or small rodents.

In solution

You can send us your antigen in solution on dry ice. We recommend limiting as far as possible the use of detergents and aggressive chemicals such as acetic acid, guanidine hydrochloride, heavy metals and other agents that are toxic to the host animal.

It is possible to immunise animals with an antigen solution containing 8M-urea, but this is more painful for the rabbits. For this reason, we ask our customers to send us the antigen as concentrated as possible so that we can dilute the solution before injection in order to decrease the final urea concentration. Antigens in solution should be sent in a volume not exceeding the authorised amount per injection.

For ex:

Rabbit	500 µL/injection
Rat	250 µL/injection
Guinea Pig	250 µL/injection
Mouse	150 µL/injection
Hen	500 µL/injection
Goat/Sheep	100 µL/injection

Note:

*Please note that the molecular mass of haptens is usually too low to elicit an immune response. Therefore, they should be provided under a format with a higher MM if Ab production is requested (e.g. coupled to a carrier).

ANIMAL WELFARE

Animal welfare is our priority, that's why we strictly follow and even exceed the sternest ethical legislation in force. We respect the 3Rs philosophy. Reduce the number of animals used. Refine space to improve animal comfort. Replace animal use by other techniques whenever possible. With this aim, we can produce polyclonal antibodies from chicken eggs, and produce monoclonal antibodies exclusively *in vitro*.

Our animal facilities are governed by stringent practices:

- BELAC, one of the most stringent ethical legislations in force.
- Facilities are cleaned 3-5 times a week with cleaning agents free from formaldehyde.
- Flow of traffic from the cleanest to the dirtiest area prevents cross-contamination.
- Daily observation of sentinel animals and health status are monitored as per FELASA recommendations.
- Each Animal has a balanced diet and the food is quality controlled.
- Temperature, pressure, relative humidity and ventilation systems (100 % HEPA filtered air for SPF animals) are recorded in real time.
- 24 hour staffed facilities with an intrusion alarm system, armoured doors and surveillance camera ensure absolute security.

ANYTIME
EXPERTS CAN VISIT OUR
FACILITIES AND AUDITS
CAN BE ORGANISED.



LARGE SCALE ANTIBODY PROJECTS

Species	Mean housing capacity
Rabbits	15000*
Guinea pigs	300*
Hamsters	200*
Rats	200*
Mice	400*

A polyclonal programme has an average yield of 10-20 mg specific rabbit Ab.

*Can be easily adapted in large customer's requests.

HOW TO ORDER

ONLINEWWW.EUROGENTEC.COM

CUSTOM ANTIBODY

Online forms are available on our web site:

1. Fill in the request form
2. Receive a quotation (and recommendations for the peptide sequence in case of anti-peptide production)
3. Complete and send the order form (you will receive the link by email with your quotation)
4. Receive a confirmation email (at this time, your immunisation will start)

For a general discussion about your project or a tailor-made quotation, please contact your sales representative or send your request to proteomics.services@eurogentec.com

For monoclonal antibodies programme, please contact: monoclonals@eurogentec.com

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Antibodies

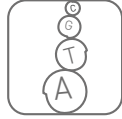
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