

Antibodies

For Life Science Research

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



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

Monoclonals

Additional **Therapeutics**

Primary and secondary

⇒ p7	Custom antibodies
⇒ p8	Host selection
⇒ p9	Polyclonal antibodies
⇒ p14	Monoclonal antibodies
∌ p16	Genetic immunisation
⇒ p17	Therapeutics
⇒ p19	Additional services
⇒ p21	Catalogue antibodies
⇒ p22	Primary
⇒ p23	Secondary

Annexes

∌ p24	Ab titer evolution
∌ p24	Bleed volumes
⇒ p25	How to provide my antigen
∌ p26	How to order
∌ p26	Shipping fees
⇒ p27	Animal welfare
⇒ p27	Related products
⇒ p27	License statements

YOUR ANTIBODY SOLUTION



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.

- 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
- · Pathogenic antigens are handled with the appropriate precautionary measures in the biosafety level 2 zone of Eurogentec's facilities.

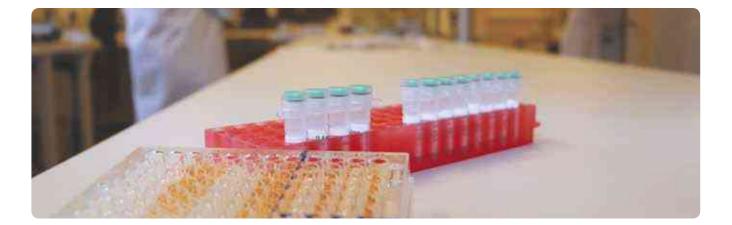
- Our proprietary Speedy 28-Day polyclonal programme reduces the immunisation time to 28 days. It uses a combination of non-Freund's adjuvants that stimulates the host's immune response and generates a high IgG/IgM ratio.
- · For difficult antigens, Eurogentec generates antibodies using genetic immunisation.

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
- 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_uH and mAb fragments, which can be produced under ISO 13485 and GMP standards.







WHAT IS MY TARGET?

POPULAR

specific Ab

SPECIFIC

CATALOGUE Ab Is there a catalogue Ab NO available? YES Order Online The Ab I test the Ab I need more perfectly suits

4643533

4648523

MARTAF

ALABERS.

4642586

45445784

MAKE SE

ACABLE S

AGNEST BLY

442489

ACIZYES

4642548

1641548

9646E W.S.

SHEEFIR

4642543

Staphofoke

Strategie

AGCESSON.

4642550

my application

My Antigen is complex and I need to target a specific epitope

CUSTOM Ab

I need a host that is not mouse or rabbit*

I need batch to batch reproducibility

I choose monoclonal Ab

I choose polyclonal Ab

Do I have my antigen (peptide, protein, cell extract, DNA ...)?

YES

I send it to **Eurogentec**

NO Eurogentec synthesises it for me

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



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

Custom antibodies

produced by Eurogentec. We are experts in the generation of polyclonal (pAb) and monoclonal (mAb) antibodies for simple projects to large and high-demanding

We offer a continuum of production capabilities including manufacturing for therapeutic applications. We can produce your recombinant Ab fragment 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



STAF

BAS

DAT

RATE

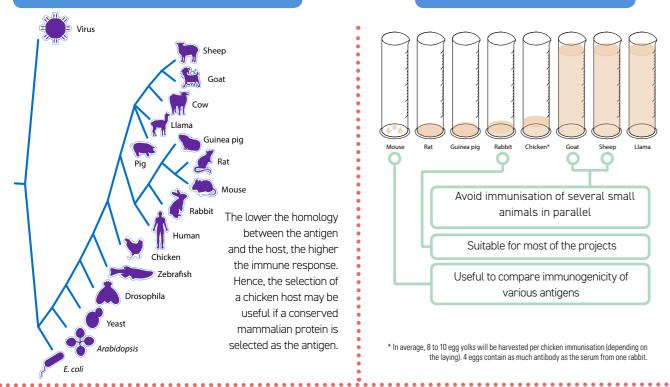
PAT

4642548

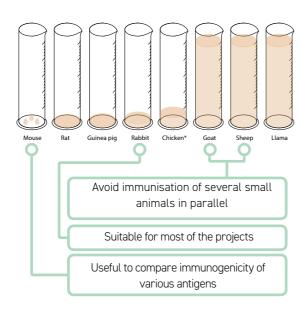
THE BEST HOST FOR YOUR PROJECT

KEY FACTORS

Antigen homology



Ab quantity



* 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.





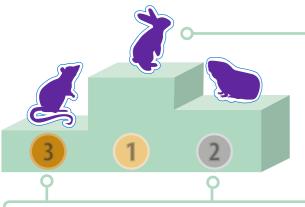
reproductibility

Therapeutics



We produce polyclonal and monoclonal Llama antibodies and collaborate with QVQ to provide V_uH 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

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

antigen either peptides or proteins.

SPEEDY 28-DAY

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

IMMUNISATION SCHEDULE



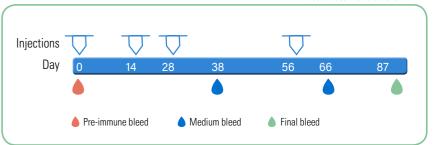
All bleeds are sent to the customer

CLASSICAL

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

IMMUNISATION SCHEDULE

All bleeds are sent to the customer

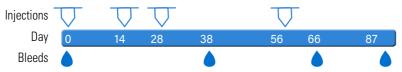


GOOD TO KNOW

** 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





Injections	∇	$\nabla\nabla$	$\overline{\bigvee}$	
Day	0	7 10	18 21	28
Bleeds			•	

		STED IN				Class	sic	al progran	nm	nes					Sp	ee	dy 28-day p	ro	grammes			
L		CT SEE PAGE 27 Sation see page 18		Anti-antigen	ļ	Anti-peptide		Double X		Anti-F	PTM*		Anti-antigen		Speedy-Mini		Anti-peptide		Double X		Anti-F	PTM*
				u provide the antigen^a eptide, protein, complex sample)		gentec designs and ynthesizes one peptide	pe	ogentec designs and synthesises two ptides to increase chances of success		Eurogenteco synthesises two pept with the PTM and	ides: one modified		ou provide the antigen^a peptide, protein, complex sample)		Eurogentec designs and rnthesises one peptide		Eurogentec designs nd synthesises one peptide	synt	urogentec designs and thesises two peptides o increase the chances of success		"Eurogentec synthesises two pept with the PTM and	ides: one modified
		Length				15-25 mg		15-25 mg each		10-20 m	ng each				10 - 16 aa		10 - 16 aa		10 - 16 aa each		10 - 13 a	aa each
	as T	Quantity	1	X		>70 %		> 70 %		>70) %		X		8-10 mg		15-25 mg		15-25 mg each		10-20 m	ng each
	Peptide	Purity				0		5 mg		5 mg	each				> 70 %		> 70 %		>70 %		> 70) %
	Pel	Coupling to a carrier (KLH, BSA, OVA, THY or MAP carrier		Q _k		5 mg		5 mg each		5 mg of mof	ied peptide		Q _k		2-3 mg to KLH		5 mg		5 mg of each		5 mg of mof	ied peptide
			Nbr	Cat#	Nbr	Cat#	Nbr	Cat#	Nbı	ır	Cat#	Nbr	Cat#	Nbr	Cat#	Nbr	Cat#	Nbr	Cat#	Nbr		Cat#
		Rabbit	2	AS-PNOR-3MORAB	2	AS-PCAP-RABBIT	2	AS-DOUB-LX	2	Phospho	AS-PSPE-CIFIC	2	AS-SUPR-ANTIGEN	1	AS-SMAF-SINGLE	2	AS-SUPR-SINGLE	2	AS-SUPR-DX	2	Phospho	AS-SUPR-PSPEC
										Acetyl Lys	AS-PTMA-LYSACET										Acetyl Lys	AS-SUPR-LYSACET
										DiAcetyl Ser	AS-PTMA -SERACET2										Citruline	AS-SUPR-CITSPEC
										Oxidized Cys	AS-PTMA-OXCYS										Oxidized Cys	AS-SUPR-OXCYS
										MonoMethyl Arg	AS-PTMA-ARGME1										MonoMethyl Arg	AS-SUPR-ARGME1
										DiMethyl Arg (sym)	AS-PTMA-ARGME2SYM										DiMethyl Arg (sym)	AS-SUPR-ARGME2SYM
										DiMethyl Arg (asym)	AS-PTMA-ARGME2ASY										DiMethyl Arg (asym)	AS-SUPR-ARGME2ASY
Ξ	Host ^b									MonoMethyl Lys	AS-PTMA-LYSME1										MonoMethyl Lys	AS-SUPR-LYSME1
satic										DiMethyl Lys	AS-PTMA-LYSME2										DiMethyl Lys	AS-SUPR-LYSME2
Immunisation										TriMethyl Lys	AS-PTMA-LYSME3											
를		Rat	2	AS-PNOR-3MORAT	2	AS-PCAP-RAT	2	AS-DOUB-LXRAT	6		AS-PSPE-CIFICRAT	2	AS-SUPR-RATANTIGEN	X		2	AS-SUPR-RATSINGLE	2	AS-SUPR-RATDX	6	on request	
		Guinea pig	2	AS-PNOR-3MOGPG	2	AS-PCAP-GUIPG	2	AS-DOUB-LXGUI	6	Phospho	AS-PSPE-CIFICGP	2	AS-SUPR-GPANTIGEN	X		2	AS-SUPR-GPSINGLE	2	AS-SUPR-GPDX	6	Phospho	AS-SUPR-PSPECGP
		Goat	1	AS-PNOR-3MOGOAT	1	AS-PCAP-GOAT	1	AS-DOUB-LXGOAT	X			1	AS-SUPR-GOATAG	X		1	AS-SUPR-GOATSING	1	AS-SUPR-GOATDX	X		
		Mouse	3	on request	3	AS-PCAP-MOUSE	3	on request	X			X		X		X		X		X		
		Chicken	2	AS-PNOR-3MOHEN	2	AS-PCAP-HEN	2	AS-DOUB-LXHEN	2	Acetyl Lys	AS-PTMA-LYSACETHEN	X		X		X		X		X		
	E	Big animal (pig, sheep, cow,)	1	on request	1	on request	1	on request	X			X		X		X		X		X		
									_	More modificat	ion on request			<u> </u>							More modificat	ion on request
	ELISA			×		Q		Q		Q	*		×	No R	Specific response at 1/20000 garantee ^c esult, No Serum, No Invoice ^d	a	Specific response t 1/20000 garantee ^c lo Result, No Serum, No Invoiced	·	cific response at 1/20000 garantee ^c at least for one of the 2 peptides) ^d		Q	
	5	Affinity Purification		Q r		C _k		Q _r		Double purificatio	n of 50 mL serum		C	Pı	urification of 10 mL Serum		C		C		Double purificatio	n of 50 mL serum
•	ody ssinį	QC validation		Q		Q _k		Q		ELISA and	SDS-PAGE		Q		ELISA		ELISA		ELISA		ELISA and	SDS-PAGE
	Antibody Processing	Delivery	Pur	Serum ified antibody (Optional)	(Op	Serum 'urified antibody otional) Remaining peptide	l (0)	Serum Purified antibody ptional) Remaining peptide		Purified a Remainin Remaining	ig serum	Pu	Serum urified antibody (Optional)		Serum Purified antibody		Serum fied antibody (Optional) Remaining peptide		Serum ified antibody (Optional) Remaining peptides		Purified a Remainin Remaining	ig serum

 \times = not applicable \Rightarrow = Optional

a. See the section "How to provide my antigen" on p.25 for more details

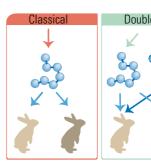
b. Other hosts are available on request

c. The guarantee only applies for peptides when approved by Eurogentec

d. The guarantee does not apply for Goat immunisation

*Post Translational Modification

Double X programmes



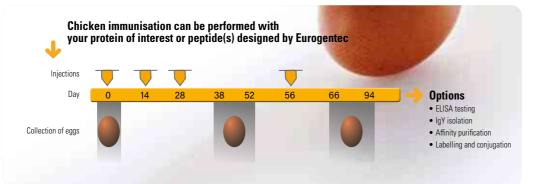
Double X programmes increase the chances of success by using two peptides corresponding to distinct regions of the targeted protein. Each host will receive a combination of the two peptides.





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. ■

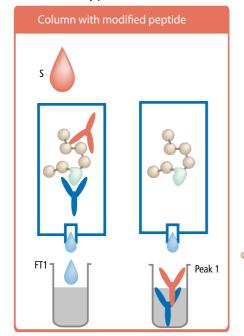
Phospho-Acethyl-DiAcetyl-MonoMethyl-DiMethyl-TriMethyl-Oxidised-Citruline-

And more on request

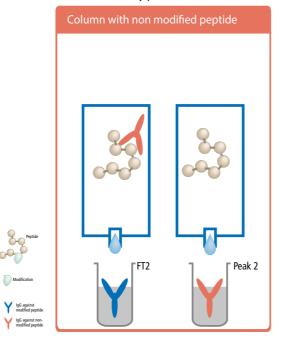
BE AWARE OF

The purfication 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.

Affinity purification 1



Affinity purification 2



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
- ELISA testing of the purified pAb against the non-PTM and PTM peptides
- Shipping



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 preimmune 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

you are a research laboratory or a pharmaceutical company.

BENEFITS

In vitro production

more countries now prohibit the mAb production in ascites, a method associated with animal pain and distress. Prior to this recommendation, Eurogented already switched to the in vitro production of mAbs bioreactors (>1000 L). ■

HOW TO START

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.

You receive

hybridomas

HYBRIDOMA GENERATION

Eurogentec develops murine hybridomas in 16-19 weeks following a stepwise approach with testing sample phases. A reporting is sent after each phase and 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:

CUSTOMER REQUEST

* Your purified protein

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

PHASE 1: IMMUNISATION PHASE 2: FUSION/HYBRIDOMA PRODUCTION

Immunisation of 4 mice (6 weeks)

ELISA report against the supplied antigen and serum sample

ELISA titer

2 - 3 weeks Myeloma Immortality Property Specific Antibody Hypoxanthine Hybridoma Secreting Cells Phosphoribosyl lg positive Transferase (HPRT) negative, Ig negative Spleen cell + Myeloma cell Sp2 OAg 14 You receive: Fusion report

Hybridoma

4 - 5 weeks Lymphocytes die Only hybridomas survive Hypoxanthine off on their own Aminopterin Thymidine (HAT) over time treatment kills off excess myeloma cells

PHASE 3: SCREENING FOR POSITIVE HYBRIDOMAS

Each cell potentially produces a different monoclonal. **Screen** wells for positive hybridomas by ELISA*

You receive: Screening report, ELISA against your antigen, Delivery of >1 ml positive supernatants (Maximum 30).

ELISA titer YES

PHASE 4: CLONING AND ISOTYPING **OF POSITIVE HYBRIDOMAS**

Cloning by serial dilution to get one cell type per well. Test each well to find desired positive clones and then isotype.

Hybridomas ELISA and isotyping report, Delivery of > 10 ml of supernatant from 1-2 positive clones, Frozen hybridomas.

4 - 5 weeks

IN VITRO PRODUCTION

STARTING MATERIAL



Your hybridoma (coming from you or from Eurogentec)



YES Production

> 200 mg

TEST PHASE Assessment of the clone productivity

MAB PRODUCTION Load Inoculum or Add Displacement Mediun Harvested Concentrated In bioreactors In CL1000 In roller bottles

YES

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.)

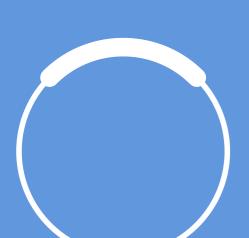




You receive

vour

^{*} 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.



Genetic immunisation

steps. The available hosts for genetic immunisation are mouse and rabbit.

Therapeutics

developed proprietary plasmids to produce GMP antibody fragments. In parallel, we collaborate with QVQ. Together we immunise llamas and generate custom llama V, H. ■



■ IN 4 STEPS





4 mice or rabbit (on request)



GENE SYNTHESIS OPTIMISATION

CLONING

Representation

of NH373 cells

on their surface

the antigen booster

fusion protein. ■

expressing

- DNA synthesis and cloning into a proprietary vector containing the booster sequence encoding a membrane protein
- DNA sequencing
- Small scale plasmid production
- Transient transfection of NIH3T3 cells
- Analysis of the surface expression of the antigen by FACS using antibodies against the booster sequence

DNA IMMUNISATION TECHNOLOGY

IMMUNISATION

- Large scale plasmid production
- Immunisation of animals
- Analysis of the sera (polyclonal antibodies) by FACS using cells transfected by the vector encoding the antigen sequence

POLYCLONAL AB

Receive polyclonal (complete serum)

> OR CONTINUE WITH THE DEVELOPMENT OF MONOCLONAL ANTIBODIES

MONOCLONAL ANTIBODY

■ 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. Please contact info.biologics@eurogentec.com if you are interested.

All antibody fragments can be produced:

- Fabs
- Nanobodies
- scFv
- · And more

Pascal Bolon

p.bolon@eurogentec.com

GOOD TO KNOW

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

and biotechnology companies around the

BENEFITS

GMP biomanufacturing of Ab

From small to large scale

Full V,,H service from llama



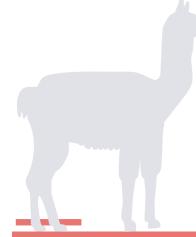
Eurogentec is an expert in protein

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



Llama antibodies and monoclonal V_uH production

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



BENEFITS

Better targeting

Easier production

Unique properties

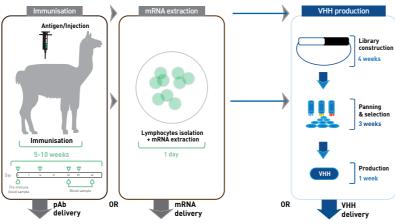
Higher stability

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

At QVQ, we generate V., H phage display libraries and perform selection and screening of high affinity monoclonal

QVQ

Eurogentec



Immunisation

- > 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).
- > 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_uH selection

The service of V_uH 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_uH 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

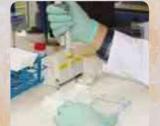
ADDITIONAL SERVICES

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

Additional

ELISA testing

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





Labelling

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

Purification

iDentification brochure). · Fluorophores are ideal for flow cytometry and fluorescent microscopy. They include AnaSpec's proprietary high-quality fluorophores HiLyte™ Fluor and CyLyte Fluor.

To avoid using a labelled

secondary antibody, your

of your choice. We offer

a large panel of dyes and

labels (refer to our Protein

specific primary antibody can

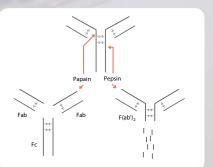
be labelled with the molecule

- 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)

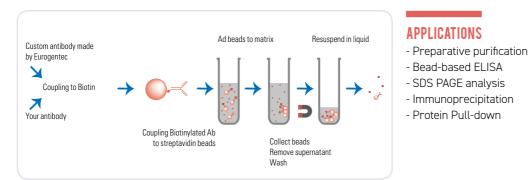


ALSO AVAILABLE

- 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. ■



A. Peptide design

P2.

P3.

P4.

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. ■

Epitope mapping

KNCSHIQPWETDCLSCLPERQDEYDPKGPK...

P1. KNCSHIQPWETDC

QPWETDCLSCLPE

CLSCLPERQDEYD

ERQDEYDPKGPK

OPTION

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

- SDS PAGE analysis

- Immunoprecipitation

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 synthetised peptides

(96 well formats), biotinylated on request

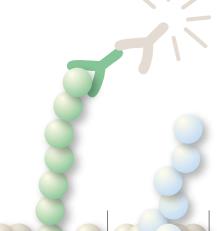
B. Library screening and epitope characterisation

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

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

1:3000 and 1:9000)



- 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

4. Epitope characterisation by ELISA

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



Catalogue antibodies

Eurogentec has always been a major European distributor of high quality catalogue antibodies recognised for its responsible and high level technical

FEATURES

Top quality antibodies

Clones with highest reputation

Key protein targets

Skilled technical support



■ PRIMARY

CHOOSE AMONG OUR FIRST-CLASS Abs

Eurogentec focuses its range of catalogue primary antibodies only on the best ones to offer you the guarantee of high quality and full satisfaction.

Best sellers

Neuronal Class III beta-Tubulin (TUJ1) Neurofilament Pan (SMI 312); Phospho

Cat #

optimab-catalog-antibodies.html ■



Product Description

Host Clone Size

Ms= mouse Rb= rabbit

VISIT OUR WEBSITE

Our range is continuously evolving http://www.eurogentec.com/

Cat #	Product Description	Host	Clone	Size
MMS-101P-XXX	OptimAb™ HA.11, mAb, purified	Ms	16B12	100µg, 500µg
AFC-101P-500	OptimAb™ HA.11, mAb, Affinity Matrix	Ms	16B12	500µg
BIOT-101L-050	OptimAb™ HA.11, mAb, Biotin Labelled	Ms	16B12	50µg
FITC-101L-050	OptimAb™ HA.11, mAb, FITC Labelled	Ms	16B12	50µg
PRB-101P-100	OptimAb™ HA.11, pAb, purified	Rb	N/A	100µg
SIG-39320-XXX	OptimAb™ Beta-Amyloid 1-16, mAb, purified	Ms	6E10	100µg 500µg
SIG-39340-100	<i>Optim</i> Ab™ Beta-Amyloid 1-16, mAb, Biotin Labelled	Ms	6E10	100µg
SIG-39220-XXX	OptimAb™ Beta-Amyloid 17-24, mAb, purified	Ms	4G8	100µg, 500µg
SIG-39240-100	OptimAb™ Beta-Amyloid 17-24, mAb, Biotin Labelled	Ms	4G8	100µg
SIG-39142-050	OptimAb™ Beta-Amyloid 1-42, mAb, purified	Ms	12F4	50µg
SIG-39142-250	OptimAb™ Beta-Amyloid 1-42, mAb, purified	Ms	12F4	250µg
SIG-39144-050	<i>Optim</i> Ab™ Beta-Amyloid 1-42, mAb, Biotin Labelled	Ms	12F4	50µg
MMS-435P-XXX	<i>Optim</i> Ab™ Neuronal Class III beta-Tubulin, mAb, purified	Ms	TUJ1	100μg, 200μg
PRB-435P-050	$\textit{Optim} Ab^{\text{TM}}$ Neuronal Class III beta-Tubulin, pAb, purified	Rb	N/A	50µg
PRB-278P-100	OptimAb™ Pax-6, pAb, purified	Rb	N/A	100µg
SIG-39138-050	$\mathit{Optim}Ab^{TM}$ sAPPbeta, pAb, purified	Rb	N/A	50µg
SMI-312P-050	$\textit{Optim} Ab^{\text{\tiny{TM}}} Pan\text{-}Axonal Neurofilament Marker, mAb, purified$	Ms	SMI-312	50µg
SMI-310P-050	$\textit{Optim} Ab^{\text{\tiny{TM}}} \text{Neurofilament H\&M Phosphorylated, mAb, purified}$	Ms	SMI-310	50µg
SMI-32P-050	$\textit{Optim} Ab^{\text{TM}} \ \text{Neurofilament H Non-Phosphorylated, mAb, purified}$	Ms	SMI-32	50µg
SMI-34P-050	$\textit{Optim} Ab^{\text{TM}} \ \text{Neurofilament H Phosphorylated, mAb, purified}$	Ms	SMI-34	50µg
SMI-31P-050	$\textit{Optim} Ab^{\text{\tiny{TM}}} \ Neurofilaments, \ Phosphorylated, \ mAb, \ purified$	Ms	SMI-31	50µg
SMI-35P-050	$\textit{Optim} Ab^{\text{\tiny{TM}}} \ \text{Neurofilaments, Hypophosphorylated, mAb, purified}$	Ms	SMI-35	50µg
MMS-126P-050	OptimAb™ RNA Polymerase II, mAb, purified	Ms	8WG16	50µg
MMS-128P-050	$\textit{Optim} Ab^{\text{TM}} \ RNA \ Polymerase \ II, \ mAb, \ purified$	Ms	CTD4H8	50µg
MMS-164P-050	OptimAb™ c-Myc, mAb, purified	Ms	9E11	50µg
MMS-150P-050	$\textit{Optim} Ab^{\text{TM}}$ c-Myc, mAb, purified	Ms	9E10	50µg
BIOT-150L-050	OptimAb™ c-Myc, mAb, Biotin labelled	Ms	9E10	50µg
SIG-39840-050	<i>Optim</i> Ab™ LRRK2, mAb, purified	Ms	MC.028.83. 76.242	50µд

υ αι π	i iodaci Description	HUSL	CIUILE	3126
SIG-39725-100	Optim Ab™ alpha-Synuclein, mAb, purified	Ms	LB509	100µg
MMS-5085-050	OptimAb™ alpha-Synuclein, mAb, purified	Ms	Syn303	50µg
SIG-39730-100	$\textit{Optim} Ab^{\text{TM}}$ alpha-Synuclein, mAb, purified	Ms	4B12	100µg
SIG-39720-100	OptimAb™ alpha-Synuclein, mAb, purified	Ms	4D6	100µg
SIG-39620-100	$\mathit{Optim}Ab^{TM}$ Prion, mAb, purified	Ms	3F4	100µg
SIG-39810-100	OptimAb™ Prion, mAb, purified	Ms	6D11	100µg
MMS-5018-050	Optim Ab™ Tau, mAb, purified	Ms	77E9	50µg
SIG-39413-050	<i>Optim</i> Ab™ Tau, mAb, purified	Ms	Tau 5	50µg
SMI-99P-100	$\textit{Optim} Ab^{\text{\tiny{TM}}} Myelin Basic Protein, mAb, purified$	Ms	SMI 99	100µg
PRB-145P-050	OptimAb™ Loricrin, pAb, purified	Rb	N/A	50µg
PRB-440P-050	$\textit{Optim} Ab^{\text{\tiny{TM}}} \ \text{Nonmuscle Myosin Heavy Chain II-A, pAb, purified}$	Rb	N/A	50µg
PRB-445P-050	$\textit{Optim} Ab^{\text{TM}} \ \text{Nonmuscle Myosin Heavy Chain II-B, pAb, purified}$	Rb	N/A	50µg
MMS-257S-050	OptimAb™ Ubiquitin, mAb, purified	Ms	PAD1	50µg
SIG-38710-100	$\mathit{Optim}Ab^TM$ P-Glycoprotein 3 (MDR3), mAb, purified	Ms	C219	100µL
MMS-106P-100	$\textit{Optim} Ab^{\text{\tiny{TM}}} \ \text{Cre Recombinase, mAb, purified}$	Ms	7.23	100µg
BIOT-106L-050	$\textit{Optim} Ab^{\textsc{tm}}$ Cre Recombinase, mAb, Biotin Labelled	Ms	7.23	50µg
PRB-106P-100	$\textit{Optim} Ab^{\text{TM}} \ \text{Cre Recombinase, pAb, purified}$	Rb	N/A	100µg
MMS-570S-050	OptimAb™ Nestin, mAb, purified	Ms	10C2	50µg
MMS-120P-100	$\textit{Optim} Ab^{\text{\scriptsize{TM}}} \ \text{Nuclear Pore Complex Proteins, mAb, purified}$	Ms	MAb414	100µg
PRB-417P-050	OptimAb™ Filaggrin, pAb, purified	Rb	N/A	50µg
SIG-3730-25	OptimAb™ Lymphatic Endothelial Marker, mAb, purified	Ms	D2-40	25μg/ 25μL
SIG-3611-25	OptimAb™ GCDFP-15, mAb, purified	Ms	D6	25μg / 25μL
MMS-162P-100	OptimAb™ Human Cytokeratin-8, mAb, purified	Ms	1E8	100µg
SIG-3465-25	<i>Optim</i> Ab™ Cytokeratin, mAb, purified	Ms	OSCAR	25μg/ 25μL
MMS-159S-100	OptimAb™ Cytokeratin-10, mAb, purified	Ms	DE-K10	100µL
MMS-130P-100	OptimAb™ AU1 Epitope Tag, mAb, purified	Ms	AU1	100µg
PRB-276P-100	OptimAb™ Pax-2, pAb, purified	Rb	N/A	100µg
BI-MECY-XXXX	OptimAb™ 5-Methylcytosine, mAb, purified	Ms	33D3	100µg 500µg 1mg

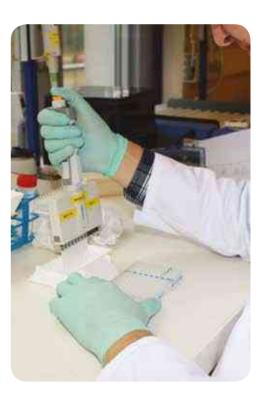
SECONDARY

CHOOSE AMONG OUR EFFICIENT SECONDARY Abs

Species

Typical Labelled with

applications



CHECK OUT OUR WEBSITE FOR A COMPLETE LISTING

₽ PROTEIN A-CONJUGATES

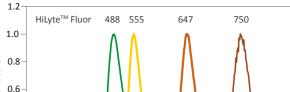
Eurogentec has developed an excellent alternative to dye labelled secondary antibodies based on the ability of Protein A to bind the FC region of most IgGs (for affinity information see p.24) This range of universal detection reagents allows the detection via streptavidin labels, or provides a super bright signal thanks to the HiLyte™ fluorophores conjugated to the Protein A.

BENEFITS

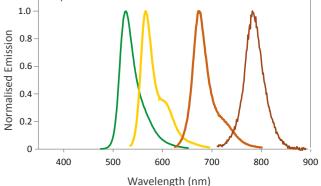
Super bright signal of the HiLyte™ Fluor

Product Name	Ex/Em (nm/nm)	Quantity	Catalog#
Protein A-HiLyte™ FLuor 488 Conjugate	499/523	1mg	AS-72235
Protein A-HiLyte™ FLuor 555 Conjugate	553/568	1mg	AS-72236
Protein A-HiLyte™ FLuor 647 Conjugate	649/674	1mg	AS-72237
Protein A-HiLyte™ FLuor 750 Conjugate	754/778	1 mg	AS-72239
Protein A-Biotin		5 mg	AS-72234

HiLyte™ Fluor absorption spectra 1.2 HiLyte™ Fluor 488 555 750 1.0 Normalised Absorbance 0.8 -0.6 0.4 -0.2 400 500 600 700 800 Wavelength (nm)



HiLyte™ Fluor emission spectra





ANNEXES

HOW TO UNDERSTAND AL TITER EVOLUTION

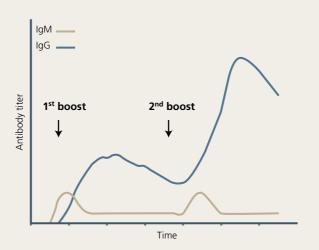
IgMs are more difficult to label and detect than IgGs because of their pentameric structure. To obtain the optimal (highest) IgG/IgM ratio, Eurogentec's immunisation programmes are optimised concerning the number and frequency of the boosts. Special attention was paid to this ratio when developing the 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.

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	volume	s			
	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



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, Belaium

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.

	injection				
Host	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			

Min antigen quantity /

400 μg 200 μg Goat Sheep 400 µg 200 µg Llama 400 μg 200 μg in 5 ml Contact us for recommendations Other

Typical antigen amounts required for immunisation in

AUTHORISED BUFFER COMPONENTS

	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 (KCI, NaCI, MgCI2)	< 1.0 M	> 1.0 M
SDS	< 2.0 %	> 2.0 %
Urea	< 6.0 M	> 8.0 M
Guanidinium HCI	-	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
lmidazole	< 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 phagocyted.

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, sylver 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, quanidine 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 250 uL/injection Rat Guinea Pia 250 µL/injection Mouse 150 μL/injection 500 μL/injection Goat/Sheep 100 μL/injection

*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).







^{*} On average, depending on the laving

HOW TO ORDER

WWW.EUROGENTEC.COM

CUSTOM ANTIBODY

Online forms are available on our web site: https://www.eurogentec.com/custom-antibodyproduction-order-forms.html

- 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

Speedy Mini Order Fo	orm.
Templatic latertain	(2) Great Manager
antibody purification with an E time Program Name	And Questimen
Pepilde Sequence (F Notion Code)	
My paptors has been approve applicable.	Ellip Curopriner and the antibody time government
amounts	

CATALOGUE ANTIBODY

The Eurogentec Ordering System (EOS) is the simplest and easiest way to place your orders.

Otherwise you can send an e-mail to order@eurogentec.com, or contact your sales representative.





SHIPPING FEES

Custom polyclonal antibodies are shipped on dry ice. Custom monoclonal antibodies are shipped on dry ice or can be lyophilised and sent at room temperature.

Catalogue antibodies are usualy shipped on dry ice. See our detailed shipping conditions on http://www.eurogentec.com/shipping-conditions.html

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.
- respect the 3Rs philosophy. Reduce the Facilities are cleaned 3-5 times a week with cleaning agents free from formaldehyde.
- number of animals used. Refine space Flow of traffic from the cleanest to the dirtiest area prevents cross-contamination.
- to improve animal comfort. Replace Daily observation of sentinel animals and health status are monitored as per FELASA recommendations.
- possible. With this aim, we can produce Each Animal has a balanced diet and the food is quality controlled.
- polyclonal antibodies from chicken eggs, and produce monoclonal antibodies

 Temperature, pressure, relative humidity and ventilation systems (100 % HEPA filtered air for SPF animals) are recorded in real time.
 - exclusively in vitro.
 24 hour staffed facilities with an intrusion alarm system, armoured doors and surveillance camera ensure absolute security.



LARGE SCALE ANTIBODY PROJECTS

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

*Can be easily adapted in large

RELATED PRODUCTS

Catalogue & custom peptides

iD gels

iD Molecular Weight Standards

iD Western 1H Detection Kits

Takyon™ qPCR kits
Test your free sample,
visit www.eurogentec.com/qpcr-takyon.html

Custom genes
Custom proteins

iD SensoLyte® assays kits

TRADEMARKS AND LABELS

EGTTM is a trademark of Kaneka Eurogentec S.A. Eurogentec® is a registered trademark of Kaneka Eurogentec S.A. HiLyteTM is a trademark of Anaspec, Inc.

LICENSE STATEMENTS

January 2018

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

rabbit Ab

HiLyte™ Fluor dyes

Use of this product is covered by one or more of the following US patents owned by AnaSpec, Inc.: USP 7,465,810, USP 7,754,893, USP 7,820,783, USP 8,258,292, and divisionals, continuations, continuations-in-part, reissues, substitutes, and extensions thereof. The purchase of this product conveys to the buyer a limited, non-exclusive, nontransferable right (without the right to sell, repackage, or further sublicense) under these patent rights to use only the amount of product purchased for the purchaser's own internal research. No other license is granted to the buyer whether expressly, by implication, by estoppel or otherwise. In particular, the purchase of this product does not include nor carry any right or license to use, develop, or otherwise exploit this product commercially, and no rights are conveyed to the buyer to use the product or components of the product for any other purposes, including without limitation, provision of services to a third party, generation of commercial databases, or clinical diagnostics or therapeutics. This product is for research use only. For information on purchasing a license to this product for purposes other than research, contact licensing@eurogentec.com.





www.eurogentec.com

Antibodies

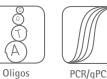
Contact: For more information, please contact your Sales representative or contact us at: proteomics.services@eurogentec.com

Toll free number: 00 800 666 00 123

Eurogentec 2018 Follow us on

















Proteins

