

# Antibodies

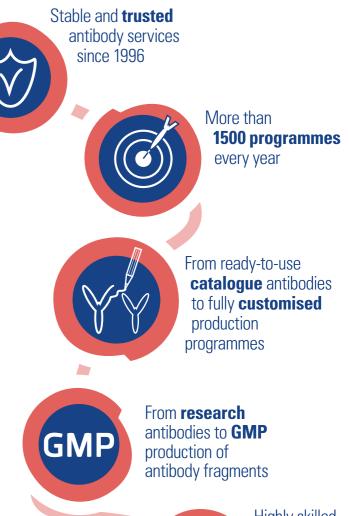
For Life Science Research

- > 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.





Highly skilled project managers dedicated to your project



## **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.

## Antigen

- DNA.

#### Immunisation

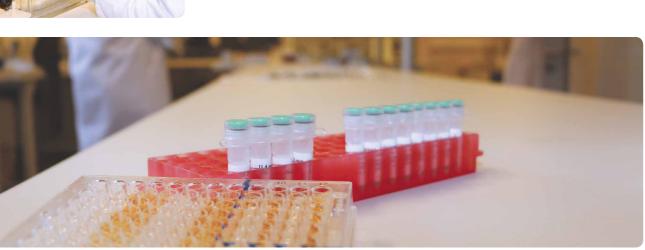
#### Antibody

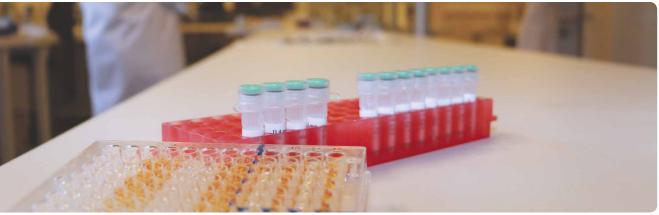
- antibodies.











Eurogentec Antibodies				
Custom	Catalogue			
Polyclonals Monoclonals Therapeutics Additional services	Primary and secondary			

<mark>⇒</mark> p7	Custom antibodies
<b>∋</b> p8	Host selection
<b>⇒</b> p9	Polyclonal antibodies
<b>∋</b> p14	Monoclonal antibodies
<b>∋</b> p16	Therapeutics
<b>∋</b> p19	Additional services
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<b>∌</b> p22	Primary
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## **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.

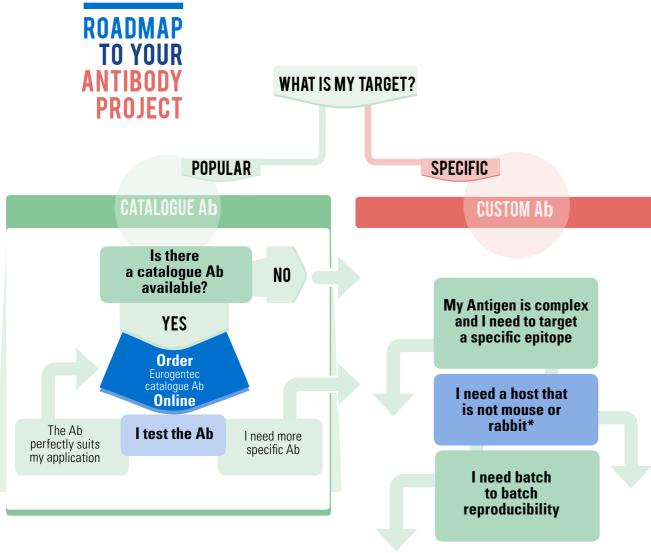
Eurogentec has distributed ready-to-use catalogue Abs for many years. Based on this long-standing expertise, we now offer a new range of top-quality primary

• 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.

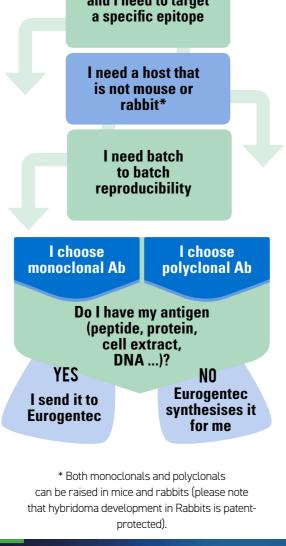
Eurogentec uses the largest animal facility in EU allowing us to manage numerous, -small to very large- antibody projects.

+ For the rapeutics applications, we provide custom  $V_{\mu}H$  and mAb fragments, which can be produced under ISO 13485 and GMP standards.











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

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.



# Custom antibodies

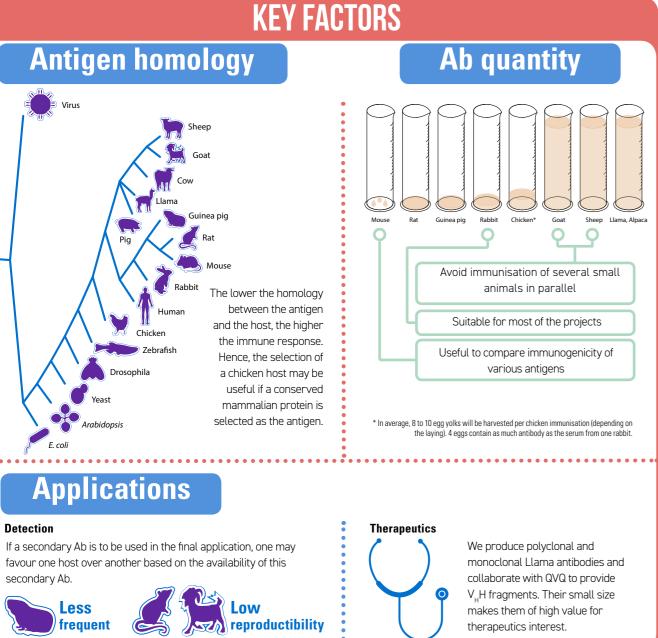
produced by Eurogentec. We are experts in the generation of polyclonal (pAb) and monoclonal (mAb) antibodies for simple projects to large and high-demanding

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.



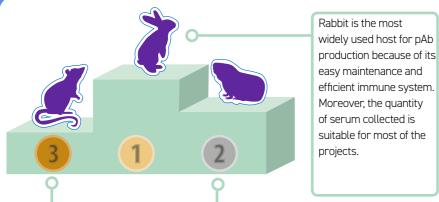






If a secondary Ab is to be used in the final application, one may favour one host over another based on the availability of this secondary Ab.

## Usage



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



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

# Custom polyclonal antibodies

## SPEEDY 28-DAY

Recommended for

- > A strong and quick response
- > An anti-PTM programme

response.

> A quick access to a small quantity of purified and ready to use pAb (with the

Immunisation lasts from 65 to 90 days

depending on the host and the immune

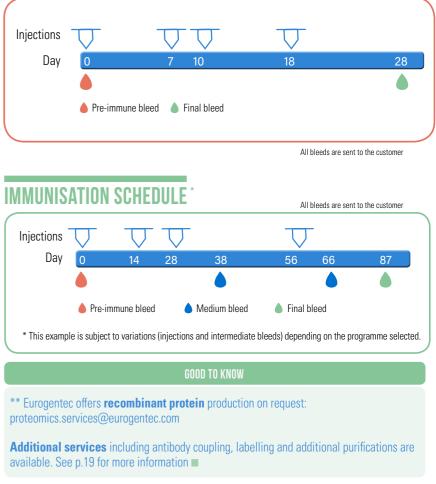
Injections

Day

Injections Day

- antigen either peptides or proteins.

## **IMMUNISATION SCHEDULE**



Eurogentec 9

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	-	Goat	1	AS-PNOR-3MOGOAT	1	AS-PCAP-GOAT		AS-DOUB-LXGOAT	X		1	AS-SUPR-GOATAG	X		1	AS-SUPR-GOATSING	1	AS-SUPR-GOATDX	X		
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 $\times$  = not applicable  $\clubsuit$  = Optional

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

b. Other hosts are available on request

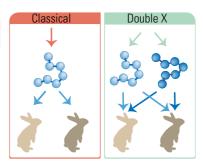
c. The guarantee only applies for peptides when approved by Eurogentec

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

Double X programmes



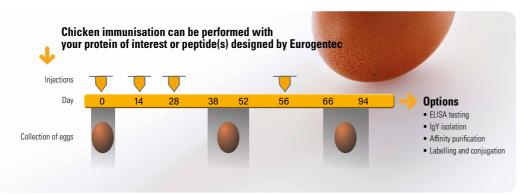
Eurogentec antibodies

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.

Eurogentec

## 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.  $\blacksquare$ 



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)

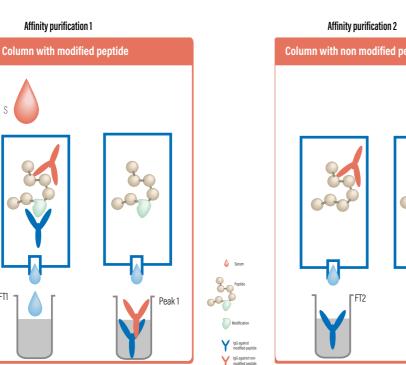
Thanks to our strong expertise in producing antibodies against PTMs, we can target classical modifications as well as more complex ones. The Speedy 28-Day programme is particularly adapted for the generation of antibodies against PTM due to the high frequency of the injections.

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

And more on request

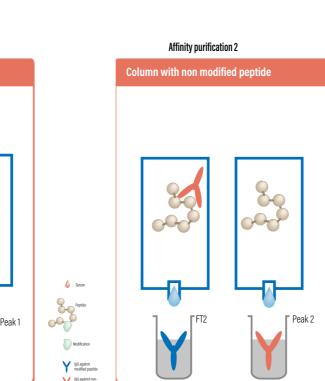
#### **BE AWARE OF**

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.



#### 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



## **1. Screening**

## 2. ELISA

- Pre-immune sera Small bleed
- Large bleed

#### Against

- The free peptide
- The carrier protein

NOTE \* In average, 8 to 10 egg volks will be harvested per chicken immunisation (depending on the laving).

# **Options** for your polyclonal programme

#### **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.

#### 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:

Positive and negative controls.



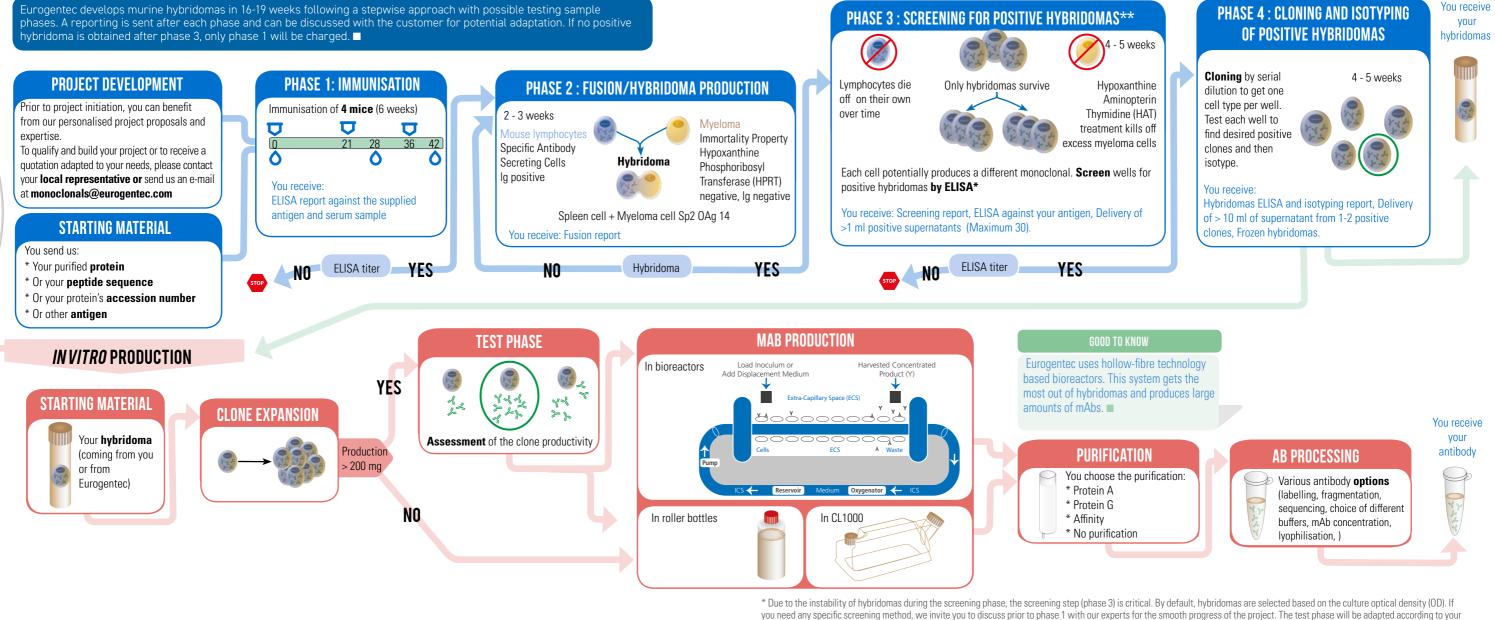


you are a research laboratory or a pharmaceutical company.

## **BENEFITS**

## In vitro production

#### **HYBRIDOMA GENERATION**



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

**CUSTOMERREQUEST** 

#### Eurogentec antibodies

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 SEE PAGE 20

#### 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.





# 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. 📃

## ⇒ 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

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

rom small to large scale

Full V, H service from llama

## Camelid antibodies and monoclonal $V_{\mu}H$ 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<sub>u</sub>H production including lymphocyte isolation and mRNA extraction. Eurogentec can label your V\_H fragments on request.

BENEFITS

Better targeting

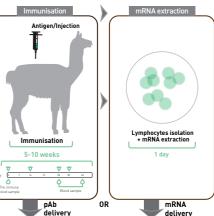
Easier production

Unique properties

/<sub>H</sub>H have a high tissue penetration and are cleared from circulation

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

#### Eurogentec



#### 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:

Higher stability V"H are stable under extreme pH,

the programme can

be stopped here and you receive pAb (crude serum or purified pAb).

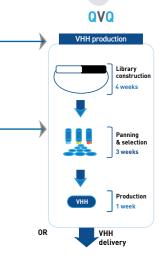
At QVQ, we generate V<sub>..</sub>H phage display libraries and perform selection and screening of high affinity monoclonal V<sub>u</sub>H.



#### **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<sub>u</sub>H selection

The service of V., H generation includes one or several of the following steps:

- > Library construction
- Deliverables: two libraries with size  $>10^7$  different clones and >90% insert.
- > Selection and screening for high affinity  $V_{\mu}H$  clones + sequence determination
- Deliverables: the sequences of at least 6 binders from 2 families.
- > Production, V<sub>H</sub>H 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<sub>µ</sub>H sequence for production, physical and "in product" stability and various types of clinical use.
- 3. Formatting of the V<sub>u</sub>H in bivalent, bispecific or custom made formats.
- 4. V<sub>u</sub>H can be custom labelled.



## Anti-llama secondary antibodies

Camelids produce 3 types of Immunoglobulins G (IgGs). Only sub-classes IgG2 and IgG3 are single chain antibodies.

To facilitate the detection and the isolation of single chain Ab (IgG 2/3) from IgG1, Eurogentec offers anti-llama secondary antibodies labeled with biotin, R-PE or unlabeled. We also offer custom labeling on request.

VHHs can be detected using a rabbit anti-VHH polyclonal Ab which is broad VHH-specific, as it binds to and allows for the detection of VHHs from different germline sequence families. Custom labeling is available on request.

Product name	Size	Ref.
Mouse anti-Ilama IgG1, mAb (Clone 27.7)	0.1 mg (0.5 mg/ml)	EG-10001-100
Mouse anti-Ilama IgG1, mAb Biotin-Iabeled (Clone 27.7)	0.1 mg (0.5 mg/ml)	EG-10001-BIOT
Mouse anti-Ilama IgG1, mAb R-PE-labeled (Clone 27.7)	0.05 mg (0.5 mg/ml)	EG-10001-RPE
Mouse anti-Ilama IgG2/3, mAb (Clone 17.11)	0.1 mg (0.5 mg/ml)	EG-10002-100
Mouse anti-Ilama IgG2/3, mAb Biotin-Iabeled (Clone 17.11)	0.1 mg (0.5 mg/ml)	EG-10002-BIOT
Mouse anti-Ilama IgG2/3, mAb R-PE-labeled (Clone 17.11)	0.05 mg (0.5 mg/ml)	EG-10002-RPE
Rabbit anti-Llama VHH pAb	25 µg (1 mg/mL)	EG-VHH001-0025
Rabbit anti-Llama VHH pAb	100 µg (1 mg/mL)	EG-VHH001-0100
Rabbit anti-Llama VHH pAb	250 µg (1 mg/mL)	EG-VHH001-0250
Rabbit anti-Llama VHH, pAb, HiLyte™ Fluor 488 labeled	0,1 mg (0,5 mg/ml)	EG-VHH001-HF488



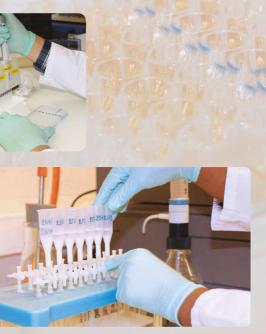
A S

**ELISA** testing

#### Purification

Eurogentec can assay your antibody in various applications including ELISA and western-blot. ■ We can perform purification of total IgG (IgY in chicken)

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



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# Additional Services

#### 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.

#### ALSO AVAILABLE

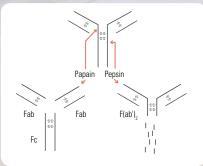
- Secondary antibodies
- Protein A coupled
- to fluorescent
- dyes and biotin, see p. 23

#### 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. ■

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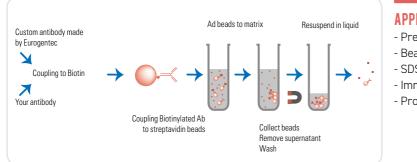
Please note that chicken IgY do not bind to the Fc receptors expressed on the surface of mammalian cells, hence generating less background (see p.12)





#### **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.  $\blacksquare$ 



### APPLICATIONS

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

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

### A. Peptide design

- KNCSHIQPWETDCLSCLPERQDEYDPKGPK ....
- P1. KNCSHIQPWETDC
- QPWETDCLSCLPE P2.
- P3. CLSCLPERQDEYD
- ERQDEYDPKGPK P4.

#### You provide

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

#### (7 aa overlaps are suggested)

#### 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

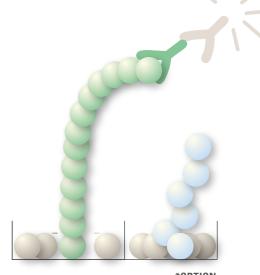
You receive

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

Assistance with the peptide design

(96 well formats), biotinylated on request

• The synthetised peptides



**\*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)

## OPTION Synthesised peptides

#### biotinvlated to assure better and oriented fixation onto the support.

(≥10 aa) can be





# standards.

## FEATURES

**Key protein** targets





# Catalogue antibodies

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

Top quality antibodies **Clones** with highest reputation

**Skilled** technical support



## 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.

#### VISIT OUR WEBSITE

Our range is continuously evolving http://www.eurogentec.com/ optimab-catalog-antibodies.html

Cat #

#### Best sellers

- β-Amvloid 1-42 (clone MM26-2.1.3) Tau (phosphorylated and controls

Size

50 µg

50 µg

5-Methylcytosine is a key regulator of gene transcription; it is closely associated to cancer as promoters of specific tumor suppressor genes are found hypermethylated; clone 33D3 is the most used and published mAb used to target 5-methylcytosine in multiple applications.



 $\beta$ -Amyloid 1-42 is a hallmark of Alzheimer's diseases, as it aggregates in the brain of diseased patients;

clone MM26-2.1.3 do not cross-react with shorter amyloid fragments



Mef2A is highly studied in differentiation models such as the zebrafish as it mediates

cellular functions in skeletal and cardiac muscle development, but is also involved in neuronal differentiation and survival

Cat #	Anti-tag Abs	Siz
AS-29779	Anti-GFP Tag, rabbit pAb	100
AS-29531-AMCA	Anti-GST Tag, mouse mAb / AMCA	100
AS-29531-BIOT	Anti-GST Tag, mouse mAb / Biotin	100
AS-29531-FITC	Anti-GST Tag, mouse mAb / FITC	100
AS-29531-H488	Anti-GST Tag, mouse mAb / HiLyte™ Fluor 488	100
AS-29531-H555	Anti-GST Tag, mouse mAb / HiLyte™ Fluor 555	100
AS-29531-H647	Anti-GST Tag, mouse mAb / HiLyte™ Fluor 647	100
AS-29531-TAMR	Anti-GST Tag, mouse mAb / TAMRA	100
AS-72250	Anti-GST Tag, mouse mAb / Europium	50 µ
AS-61250	Anti-His Tag, mouse mAb / non labeled	50 µ
AS-61250-AMCA	Anti-His Tag, mouse mAb / AMCA	100
AS-61250-BIOT	Anti-His Tag, mouse mAb / Biotin	100
AS-72251	Anti-His Tag, mouse mAb / Europium	50 µ
AS-61250-FITC	Anti-His Tag, mouse mAb / FITC	100
AS-61250-H488	Anti-His Tag, mouse mAb / HiLyte™ Fluor 488	100
AS-61250-H555	Anti-His Tag, mouse mAb / HiLyte™ Fluor 555	100
AS-61250-H647	Anti-His Tag, mouse mAb / HiLyte™ Fluor 647	100
AS-61250-TAMR	Anti-His Tag, mouse mAb / TAMRA	100
Cat #	Zebrafish-specific Abs	Siz
AS-55811	Anti-MBP (IN), Z-Fish™, rabbit pAb	250
AS-55818	Anti-Nes (CT), Z-Fish™, rabbit pAb	250
AS-55339	Anti-Actin-beta (CT), Z-Fish™, rabbit pAb	250
AS-55609	Anti-Mef2-pan (IN), Z-Fish™, rabbit pAb	250
AS-55533	Anti-NeuroD (CT), Z-Fish™, rabbit pAb	250
AS-55421	Anti-PCNA (IN), Z-Fish™, rabbit pAb	250
AS-55877	Anti-Vegf-c (IN), Z-Fish™, rabbit pAb	250
AS-55383	Anti-TNF-a (IN), Z-Fish™, rabbit pAb	250
AS-55345	Anti-MMP-9 (hinge), Z-Fish™, rabbit pAb	250
AS-55715	Anti-Cdh-5 (IN), Z-Fish™, rabbit pAb	250
AS-55600	Anti-ItgA-2b (IN), Z-Fish™, rabbit pAb	250
AS-55712	Anti-CD4 (NT), Z-Fish™, rabbit pAb	250
AS-55713	Anti-CD8-a (CT), Z-Fish™, rabbit pAb	250
/10/00/10		050
AS-55917	Anti-MPEG-1 (CT), Z-Fish™, rabbit pAb	250
	Anti-MPEG-1 (C1), Z-Fish <sup>™</sup> , rabbit pAb Anti-Mpx (NT), Z-Fish <sup>™</sup> , rabbit pAb	250 250

## SECONDARY

## CHOOSE AMONG **OUR EFFICIENT SECONDARY Abs**



## ■ 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<sup>™</sup> fluorophores conjugated to the Protein A.

#### **BENEFITS**

Super bright signal of the HiLyte™ Fluor Suitable for multiple detection applications arge panel of emission wavelengths

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

#### Highlight on key Best sellers BI-MECY-0100 Anti-5-Methylcytosine, mouse mAb clone 33D3 100 µg Anti-5-Methylcytosine, mouse mAb clone 33D3 BI-MECY-0500 500 µg Anti-5-Methylcytosine, mouse mAb clone 33D3 BI-MECY-1000 1000 µg AS-29553 Anti-Actin, a smooth muscle (h,m), rabbit pAb non labeled 50 µg AS-29553-AMCA Anti-Actin, a smooth muscle (h,m), rabbit pAb AMCA 50 µg AS-29553-BIOT Anti-Actin, a smooth muscle (h,m), rabbit pAb Biotin 50 µg AS-29553-FITC Anti-Actin, a smooth muscle (h,m), rabbit pAb FITC 50 µg AS-29553-H488 Anti-Actin, a smooth muscle (h,m), rabbit pAb HiLyte™ Fluor 488 50 µg AS-29553-H555 Anti-Actin, a smooth muscle (h,m), rabbit pAb HiLyte™ Fluor 555 50 µg 50 µg AS-29553-H647 Anti-Actin a smooth muscle (h m) rabbit nAb Hillyte™ Fluor 647 AS-29553-TAMR Anti-Actin, a smooth muscle (h,m), rabbit pAb TAMRA 50 µg 50 µg AS-29588 Anti-CD3-ensilon (h) rabbit nAb AS-55043A Anti-mouse P2Y12, rabbit pAb 50 µg AS-54819 Anti-CD45R (m), rat mAb clone RA3-6B2 50 µg AS-54487 Anti-Nephrin CT (h,m,r), rabbit pAb 50 µg AS-28139 Anti-SOX9 Ser181 (h.m), rabbit pAb 50 µg AS-29577 Anti-MMP-7/Matrilysin, CT (h,m), rabbit pAb - 50µg 50 µg Anti-β-Amyloid (1-40) (h,m,r), mouse mAb clone MM32-13.1.1 AS-55921 50 µg AS-55922 Anti-β-Amyloid (1-42) (h,m,r), mouse mAb clone MM26-2.1.3 50 µg AS-56074 Anti-β-Amyloid (1-6) (monomers, oligomers, fibrils), rabbit pAb 50 µg AS-56075 Anti-β-Amyloid (17-23) (oligomers, fibrils), rabbit pAb 50 µg AS-56076 Anti-β-Amyloid (23-29) (monomers), rabbit pAb 50 µg AS-55914 Anti-MOG (h,m,r), mouse mAb clone B1A 250 µl AS-55329 Anti-β-Synuclein (pSer 87) (h), rabbit pAb 50 µg AS-54960 Anti-Tau pThr181 (h), rabbit pAb 50 µg AS-54963 Anti-Tau pSer199/202 (h), rabbit pAb 50 µg AS-28017 Anti-Tau pSer202 h,m), rabbit pAb 50 µg AS-54965 Anti-Tau 198,199,202,205 (h,m), rabbit pAb 50 µg AS-54969 Anti-Tau 212,214,217 (h,m), rabbit pAb 50 µg AS-54968 Anti-Tau pThr217 (h), rabbit pAb 50 µg AS-55313 Anti-Tau pThr231 (h), rabbit pAb 50 µg AS-54974 Anti-Tau Ser262 (h,m), rabbit pAb 50 µg Anti-Tau pSer262 (h), rabbit pAb AS-54973 50 µg Anti-Tau Acetyl Lys280 (h,m,r,b,mk), rabbit pAb AS-56077 50 µg AS-56078 Anti-Tau Lys280 (h,m,r,b,mk), rabbit pAb 50 µg AS-54977 Anti-Tau pSer396 (h), rabbit pAb 50 µg 50 µg AS-54978 Anti-Tau pSer400 (h), rabbit pAb

Anti-Tau GlcNAc Ser400 (h,m,r,mk), rabbit pAb

Anti-Tau 409,412,413 (h), rabbit pAb

AS-55945

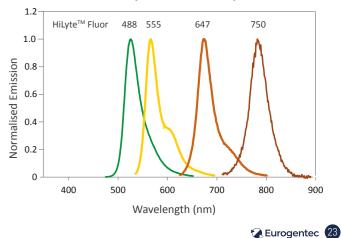
AS-55416



#### HiLyte<sup>™</sup> Fluor absorption spectra 1.2 HiLyte<sup>™</sup> Fluor 488 555 750 647 1.0 Normalised Absorbance 0.8 0.6 0.4 0.2 0\_ 400 500 600 700 800

Wavelength (nm)

#### HiLyte<sup>™</sup> Fluor emission spectra



# ANNEXES

# HOW TO UNDERSTAND

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.

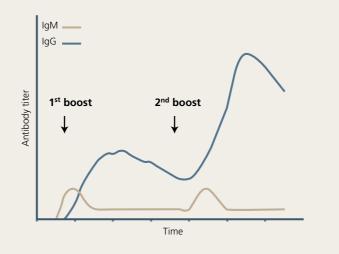
### **BLEED VOLUME** DISCOVER

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	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.

#### 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.

Min. antigen quantity / 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				
Goat	400 µg	200 µg				
Sheep	400 µg	200 µg				
Llama	400 µg	200 µg				
Other	Contact us for re	commendations				

Typical antigen amounts required for immunisation in the most cr mon 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 (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					
Imidazole	< 3 M	> 3 M					
TFA	-	High risk of toxicity					

Compounds and formulations that are acceptable for immunisation



#### **Shipping address:**

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

### **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, 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 $\mu$ L/injection
Guinea Pig	250 $\mu$ L/injection
Mouse	150 µL/injection
Hen	500 $\mu$ L/injection
Goat/Sheep	100 µL/injection





<sup>\*</sup>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).

### **HOW TO ORDER** ONLINE 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

### CATALOGUE ANTIBODY

or contact your sales representative.

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,

🃜 Cart	>	Shipping	>	Invoicing	Confirmation
My Kits & Reag	ients (2	2 item(s) / EUI	ج)		
By Family	By Refe	erence			(?) help
CATALOG ANTIBODIES	5				
DESCRIPTION			SIZE	REFERENCE	QUANTITY YOUR PRICE



Online forms available

on our website

EASY

Speedy Mini Order Form

he Speedy Mini includes your peptide

sur Program Name

Next Page

## **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**

#### Our animal facilities are governed by stringent practices:

recommendations.

- sternest ethical legislation in force. We

Animal welfare is our priority, that's why

we strictly follow and even exceed the

- animal use by other techniques whenever
- and produce monoclonal antibodies

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



## **RELATED PRODUCTS**

#### Catalogue & custom peptides

iD gels iD Molecular Weight Standards iD Western 1H Detection Kits

Takyon<sup>™</sup> gPCR kits Test your free sample, visit www.eurogentec.com/gpcr-takyon.html

Custom genes Custom proteins

iD SensoLyte® assays kits



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
- possible. With this aim, we can produce Each Animal has a balanced diet and the food is quality controlled.
- polyclonal antibodies from chicken eggs, 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 ner's request

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

## TRADEMARKS AND LABELS

HiLyte<sup>™</sup> is a trademark of Anaspec, Inc.

## LICENSE STATEMENTS

Sept.2019

HiLyte<sup>™</sup> 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-inpart, 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:

Õ

(A) Oligos

PCR/qPCR

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

Eurogentec 2019

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Peptides

Proteins

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