

Our services suitable  
to your requirements.

## Seramun Services

### Custom Antibody Production & Preparation

- high affinity  
monoclonal and  
polyclonal antibodies
- purification
- conjugation
- long years  
of experience

Seramun

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We are your partner for:

Substrates

Protein stabilizers

Antibodies

Customized research

In-vitro diagnostics

**Y**eramun  
Diagnostica GmbH



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# Customized Polyclonal

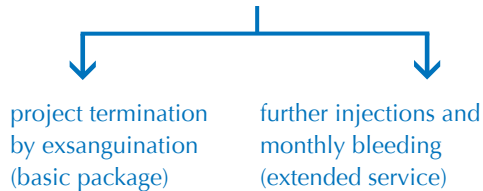
After receipt of antigen we carry out your order (for rabbits we recommend two individuals per antigen)

## Antibody Production

DEVELOPMENT OF HIGH AFFINITY POLYCLONAL ANTIBODIES BY A SPECIAL PROTOCOL AND PROCEDURE

### Procedure

1. Pre-bleed
  - 5 ml / rabbit
  - 25 ml / sheep
2. Basic injection
3. Booster injections (three times for basic package)
4. Production bleed (hyperimmune serum 1)
  - 20-25 ml / rabbit
  - 150-200 ml / sheep
5. ELISA titer testing of preimmune serum in comparison to hyperimmune serum 1
6. Shipment of preimmune serum and hyperimmune serum 1
7. Customer testing and decision for:  
either



### Time schedule

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- Week 1: • Preimmune serum  
• Basic injection
- Week 4-8: • Booster injections(1-3)
- Week 9: • Hyperimmune serum 1
- Week 10: • ELISA titer testing and shipment of serum

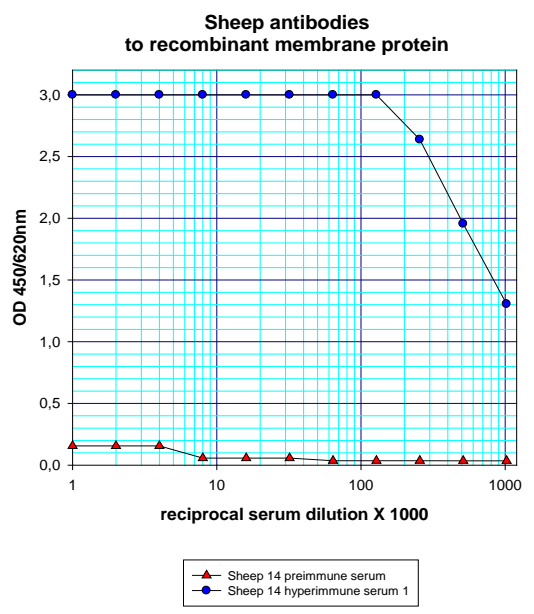
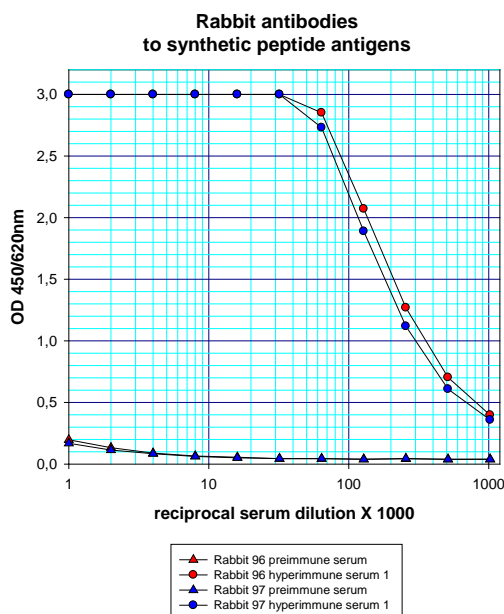
### Quality control

Comparative titration of preimmune serum and hyperimmune serum 1 according to a standardized solid-phase ELISA protocol.

### Test principle

- Solid-phase adsorbed antigen
- Serum incubation 60 min / 37 °C
- Conjugate incubation 60 min / 37 °C
- Substrate reaction 10 min / 20-25 °C
- Measurement at 450 / 620 nm wavelength

### Test example



# Customized Monoclonal Antibody Production

After receipt of antigen we carry out your order

DEVELOPMENT OF MURINE HYBRIDOMA CLONES FOR PRODUCTION OF MONOCLONAL ANTIBODIES FOLLOWS A STEP BY STEP PROTOCOL WITH THE POSSIBILITY OF PROJECT TERMINATION AT ANY STEP OF THE DEVELOPMENT

## Procedure

### Step Protocol

0. Delivery of the antigen by the customer
1. Immunization of 3 mice and bleed for control of antibody titer by solid phase ELISA; selection of one mouse for cell fusion
2. PEG mediated cell fusion of mouse splenocytes with mouse myeloma cells (SP2/0-Ag14), screening for hybridoma growth and antibody production
3. Hybridoma selection, Subcloning of 3 selected clones by limiting dilution (3X per clone), propagation and cryoconservation (5 ampoules per clone)
4. Customer decision for: either or



## Time schedule



Step 1: 12-14 weeks

Step 2: 2- 4 weeks

Step 3: 4- 6 weeks

## Additional service

Mycoplasma testing by PCR

Determination of antibody isotype and IgG sub-classes by ELISA

IgG quantification by ELISA

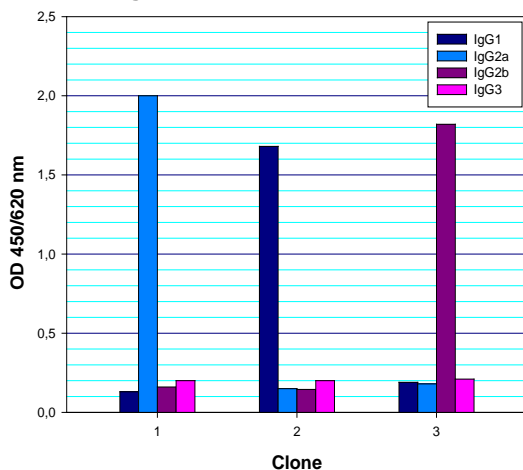
Antibody production

Antibody purification

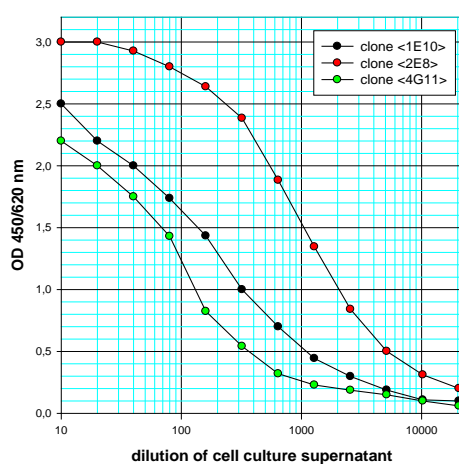
Antibody conjugation

## Test example

IgG subclass determination



Reactivity testing after subcloning



# Antibody Purification and Conjugation

OUR SERVICE IS BASED ON LONG TIME OF EXPERIENCE IN ANTIBODY PURIFICATION AND LABELLING FOR DEVELOPMENT OF IMMUNOASSAYS IN THE FIELD OF RESEARCH OR IN-VITRO DIAGNOSTICS

## Antibody purification

Purification of polyclonal antibodies from hyperimmune sera by:

- Combination of salt precipitation and ion exchange chromatography or affinity chromatography on Protein A-Sepharose with FPLC. Removal of cross reactive antibodies and antibodies emerged by impurities of the antigen by negative immunoadsorption chromatography
- Isolation of specific antibodies from the IgG fraction of hyperimmune sera by immunoadsorption chromatography with insolubilized antigen
- Purification of monoclonal antibodies from culture supernatant by affinity chromatography on Protein A- or G-Sepharose

## Analytcs

Purified antibodies are analysed by SDS Polyacrylamid Gel Electrophoresis and titration in solid phase ELISA; Antibody conjugates are analysed by Solid phase ELISA

## Antibody conjugation

We label your purified antibody with

**HRP** (RZ  $\geq 3$ ; activity  $> 250$  U/mg)  
Modified protocol of Wilson and Nakane (1978); molar ratio of 1:4

**AP** (recombinant, activity  $> 5000$  U/mg),  
molar ratio 1:4

**N-hydroxysuccinimido-Biotin**  
Modified protocol of Wilchek and Bayer (1990); molar ratio 1:30

**Fluoresceinisothiocyanat** (Isomer I)  
Modified protocol of Mohr (1991);  
molar ratio 1:8;  
determination of F/P-ratio by photometry (usually 2.5-5.0)

## Costs

Please contact [info@seramun.com](mailto:info@seramun.com) for a detailed quotation.

### Example:

affinity purified anti human IgM (goat)

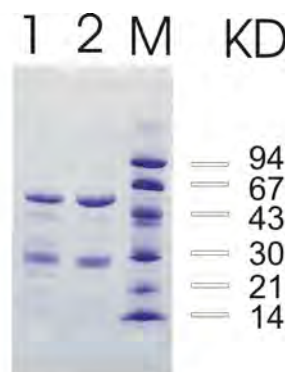
Heavy chain, 50 kD

Light chain, 25 kD

1,2: a-human-IgM

M: molecular weight marker

KD: molecular weight



Reactivity of affinity purified goat anti-human-IgM with solid-phase adsorbed human-IgG and human-IgM resp.

