hyglos
creative bioscience solutions

Application Note
LPS-free Antibodies

Protocol
LPS removal from Antibody Solutions (e.g. ***IgG)***
with EndoTrap®

Index

Introduction

Antibodies & Endotoxin Contamination

Endotoxins (LPS) are cell-associated substances that are structural components of the outer membrane of Gram-negative bacteria. Because these bacteria are ubiquitous, it is not surprising that endotoxins are frequent contaminants of biochemical preparations. Antibody solutions can be contaminated by endotoxin as can any other product. The presence of endotoxin in products for injection can result in pyrogenic responses ranging from fever and chills to an irreversible and fatal septic shock. Therefore, any drug for injection requires the demonstration of absence of LPS as part of the quality control process.

Introduction: EndoTrap®

EndoTrap® is an affinity matrix for the efficient removal of bacterial endotoxins from solutions. EndoTrap® can be employed both in batch or chromatography mode. EndoTrap® has been developed for the removal of endotoxins from aqueous solutions containing low or high molecular weight substances. Frequently, endotoxin removal from protein solutions is insufficient with standard methods including ultrafiltration, ion exchange chromatography, or two phase extraction.

The high affinity of EndoTrap® ligand to endotoxin enables the efficient capturing of endotoxins even at very low endotoxin concentrations. The EndoTrap® ligand is immobilized covalently on beaded agarose to ensure negligible leakage of EndoTrap® ligand. The endotoxin binding capacity of EndoTrap® in aqueous buffers is about 2 x 10^6 EU/ml matrix. Non specific binding of proteins to EndoTrap® is extremely low, delivering a mass yield which typically exceeds 95% (EndoTrap® blue) or 90% (EndoTrap® red). The protocol of EndoTrap® is user friendly, yields rapid results as it is designed as a flow-through system and does not need training or special equipment.

The EndoTrap® system can be reused at least three times (in general 10 times) without loss of endotoxin removal efficiency! EndoTrap® is applicable also for the downstream process as it is also available as 50% slurry. The EndoTrap® system can be reused at least three times without loss of endotoxin removal efficiency!

On **page 5-7** you find the protocol “LPS removal from antibody solutions with EndoTrap® red”. In general both EndoTrap® systems are suitable for removing LPS from antibody solutions. Which system you need depends on the buffer composition. We used EndoTrap® red as common antibody solutions are diluted in PBS buffer and EndoTrap® red is optimized for PBS buffer.

EndoTrap® blue and EndoTrap® red have similar characteristics. The products are different basically in their buffer compatibilities as the binding mechanism to LPS is different.

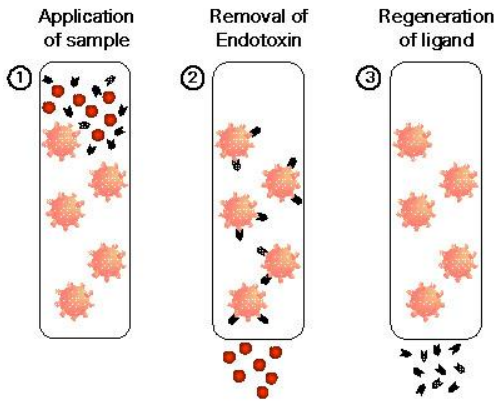
The most important differences between both EndoTrap®-family products:

	EndoTrap® blue / EndoTrap® HD	EndoTrap® red
▪ pH (buffer)	pH 4-10	pH 6-9
▪ Ionic strength	up to 600 mM NaCl	up to 250 mM NaCl ***we recommend < 100 mM NaCl***
▪ Suitable with EDTA and other Calcium chelators containing buffers	No	Yes
▪ Customer specific equilibration buffer has to be enriched with calcium	Yes	No
▪ PBS can be used as equilibration buffer	Yes, when enriched **freshly** with 50-100 µM Ca^{2+}!	Yes

General specifications of EndoTrap®

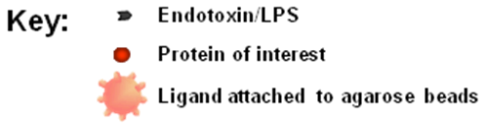
Ligand	EndoTrap® blue / HD or EndoTrap® red respectively
Binding capacity	2.000.000 EU/ml resin (each cleaning step theoretically yields a **two log reduction** of LPS)
Dissociation constant for LPS	$K_D = 5 \times 10^{-8}$ M
Support matrix	Highly cross-linked 4% **agarose**, spherical beads
Void volume	0.3 to 0.5 ml
Mean particle size	90 µm
Max. flow rate	0.2-1 ml/min
Max. pressure	**3 bar**, 43 psi, 0.3 MPa (when using automated systems)
Temperature stability	Regular use between 4°C and room temperature
Storage	At 2-8°C in regeneration buffer (RB blue or RB red) supplemented with 0.02% sodium azide. **Do not freeze!**
Shelf life	EndoTrap® is stable until the stated expiry date when stored correctly.

Principle



Principle of EndoTrap®:

1. Endotoxin-contaminated proteins and aqueous solutions are applied
2. Endotoxin is captured, proteins elute
3. Regeneration of ligand by using regeneration buffer



Kit components of EndoTrap®®

Prepacked columns			
	EndoTrap® 1/1	**EndoTrap® 5/1**	
	1 x 1 ml column, 125 ml equilibration buffer, 125 ml regeneration buffer	5 x 1 ml column, 250 ml equilibration buffer, 125 ml regeneration buffer	
Resin (50% slurry)			
	EndoTrap® 10	**EndoTrap® 50**	**EndoTrap® Bulk**
	10 ml settled resin (50% slurry), 250 ml equilibration buffer, 250 ml regeneration buffer	50 ml settled resin (50% slurry), 125 ml 10x equilibration buffer 125 ml 10x regeneration buffer	> 1 litre resin (50% slurry)
Material not provided			
	Storage buffer		
	Please add **0.02% sodium azide** to the necessary volume of regeneration buffer (RB)		

Storage

EndoTrap® is supplied as **prepacked columns** (EndoTrap® 1/1 or 5/1, 1-ml *column material*) or as **50% slurry** (EndoTrap® 10, 50 or bulk, *resin material*) in regeneration buffer (RB) supplemented with 0.02% sodium azide. EndoTrap® is stable until the stated expiry date when stored correctly. Regenerated EndoTrap® matrix should be stored at 2-8°C in regeneration buffer (RB) supplemented with 0.02% sodium azide. You can also use 20% ethanol as storage buffer; however, the storage time will then be only 4 weeks. **Do not freeze!**

Which buffer systems are compatible with EndoTrap®?

Customer specific buffers may be used for equilibration and endotoxin binding. Below you find summarized what to consider if you prefer using your own buffer:

EndoTrap® blue	EndoTrap® red
Endotoxin removal with EndoTrap® blue is efficient in the pH range of 4 - 9 and NaCl concentrations of 50 - 600 mM. Buffers such as HEPES, TRIS, MOPS, MES, and PIPES are recommended. Citrate buffers and chelators of divalent cations (like **EDTA**) **have to be avoided**. **Important:** Custom specific buffers must contain 0.1 to 1 mM Ca^{2+} or Mg^{2+}. When using **PBS buffer** always add 1 mM Ca2+ (e.g. $CaCl_2$) and 1 mM Citrate pH 7 freshly to the custom specific buffer. Otherwise phosphate and Ca2+ form an insoluble complex and will precipitate. For **DNA application** the following buffer composition is recommended: 10 mM Tris-HCl, 1 mM $CaCl_2$, 300 mM NaCl, pH 8.0	Endotoxin removal with EndoTrap® red is efficient in the pH range of 6 - 9 and NaCl concentrations in the range of 50 - 250 mM (best results with < 100 mM NaCl). Buffers such as HEPES, PBS, TRIS, MOPS, MES, PIPES and also Citrate, Acetate, Glycine and Carbonate buffers are recommended.EndoTrap® red **is suitable** with chelators of divalent cations (like **EDTA**). **Phosphate buffers with NaCl concentrations below 100 mM** are recommended for EndoTrap® red. **Important:** With a "classical" **PBS buffer** (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4), the LPS removal rate will be ~97% for each cleaning step. With a "half-concentrated" PBS buffer LPS removal rates of ~99% can be achieved. Therefore dilute the PBS buffer 1:2 with endotoxin free water (5 mM Na_2HPO_4, 0.9 mM KH_2PO_4, 68.5 mM NaCl, 1.35 mM KCl, pH 7.4).

How to calculate the number of required cleaning steps?

EndoTrap® can be reused at least three times (in general 10 times) without any loss of endotoxin removal efficiency. In case your starting endotoxin concentration is very high or in case you wish to reach a very low endotoxin concentration, EndoTrap® can be applied in a consecutive manner several times.

The 1-ml column is suitable for sample concentration in the range of 1-10 mg/ml and for a sample volume up to 50 ml. Each round of application **theoretically yields a two log reduction of endotoxin**.

However, parameters such as pH, ionic strength, temperature, contact time, etc. might have to be optimized for each application to obtain maximum endotoxin removal with minimum loss of product. Depending on your LPS starting concentration [EU] (1 EU = 100 pg LPS) you must perform a certain number of cleaning steps, in order to achieve your desired LPS end concentration [EU]. To achieve best results and to be able to calculate which package size you need for your desired performance, **total LPS units applied should not exceed 30-50% of the maximum resin capacity.**

Starting LPS concentration (buffer) [EU]	After 1. cleaning step [EU]	regeneration step (kit includes regeneration buffer RB)	After 2. cleaning step EU]	regeneration step (kit includes regeneration buffer RB)	After 3. cleaning step [EU]
100.000	1.000		10		3
10.000	100		1		0.3
1.000	10		3		0.9
100	1		0.3		0.09
10	3		0.9		0.27
1	0.3		0.09		0.027
0.1	0.03		0.009		<0.005

LPS removal from buffers: With repetitive use of EndoTrap®, you can achieve concentrations as low as 0.005 EU/ml.
LPS removal from proteins: With repetitive use ofEndoTrap®, decrease to concentrations as low as 0.1 EU/ml is possible. As it is a biological system, the efficiency ofEndoTrap® slightly decreases at low endotoxin contamination levels. At 0.1 EU/ml the removal efficiency is approximately 70%.
If you do not yield your desired endotoxin level after the third cleaning step (each cleaning step ends with a regeneration step), please contact us and use our technical support!

Comparison of the specifications of EndoTrap® blue and EndoTrap® red

	EndoTrap® blue	EndoTrap® red
Tested substances which can be applied onto the column	▪ proteins ▪ peptides ▪ antibodies ▪ **plasmid DNA**	▪ proteins ▪ peptides ▪ antibodies
Regeneration buffer (endotoxin concentration < 0.02 EU/ml)	Regeneration buffer "blue" (based on "**HEPES buffer**", pH 7.5)	Regeneration buffer "red" (based on "**Phosphate buffer**", pH7.4)
Equilibration buffer (endotoxin concentration < 0.02 EU/ml)	Equilibration buffer "blue" ("**HEPES buffer**", pH 7.5 **enriched with 0.1 mM $CaCl_2$**)	Equilibration buffer "red" ("**Phosphate buffer**", pH 7.4 **with 80 mM NaCl**)
If you want to use your **own buffer** for equilibration (instead of the kit included equilibration buffer)	We tested EndoTrap® blue successfully with **HEPES, Borate, TRIS, MOPS, MES, PIPES** when 50-100 µm Ca^{2+} were freshly added.	We tested EndoTrap® red successfully with **PBS, HEPES, Borate, TRIS, MOPS, MES, PIPES, Citrate, Acetate, Glycine** and **Carbonate buffers**
pI of applied proteins	pI from 5-9	pI from 5-9
pH (buffer)	pH 4-9	pH 6-9
Ionic strength	up to 600 mM NaCl	up to 250 mM NaCl ***we recommend < 100 mM NaCl***
Recommended working concentration of applied substances	1-10 mg/ml	1-10 mg/ml
Recommended sample volume	up to 50 ml	up to 50 ml
Tested substances which **do not interfere** with the performance of EndoTrap®*	up to 10 mM DTT (Dithiothreitol) 0.005% Tween20® max. 0.005% NaDOC max. 0.5 M GdnHCl 10% DMSO 20% Isopropanol 20% Methanol 20% Ethanol 10% Glycerol / Glycerin 0.5 M urea (up to 2 M at pH 7 possible) 300 mM Imidazol	DTT not tested max. 0.005% NaDOC 20% DMSO 20% Isopropanol 40% Methanol 20% Ethanol 20% Glycerol / Glycerin 1 M Urea 300 mM Imidazol
Tested substances which **interfere** with the performance of EndoTrap® and therefore have an **inhibitory effect** for the binding to LPS*	▪ > 10 mM NaOH ▪ SDS and other detergents ▪ Citrate ▪ EDTA, and other Calcium chelators (EGTA, HEDTA, NTA) ▪ Ammoniumsulphate	▪ > 250 mM NaCl ▪ SDS, Tween20 and other detergents ▪ GdnHCl ▪ Ammoniumsulphate
Tested kinds of LPS (bacteria strain)	▪ *Escherichia coli* K12, R1, R2, R3, R4 ▪ *Salmonella enterica* ▪ *Citrobacter freundii* ▪ *Citrobacter amalonaticus* ▪ *Citrobacter koseri* ▪ *Pseudomonas aeruginosa* ▪ *Pseudomonas stutzeri* ▪ *Enterobacter aerogenes* ▪ *Enterobacter asburiae* ▪ *Enterobacter cloacae* ▪ *Aeromonas hydrophilia*	▪ *Escherichia coli* K12 ▪ *Salmonella enterica* ▪ *Citrobacter freundii* ▪ *Pseudomonas aeruginosa* ***for*** ▪ ***Klebsiella pneumoniae*** ▪ ***Serratia marcescens*** ***we recommend EndoTrap® red!***

*Please consider that the indicated concentrations and substances refer to the performance of EndoTrap®. Some of these substances which can be used during the cleaning with EndoTrap® are **not suitable for the LAL test**. EndoLISA® endotoxin detection assay can be used as an alternative method.

Assembly of a feasibility study

As the efficiency of EndoTrap® depends on the characteristics of the used sample we recommend making a feasibility study when handling "expensive" sample or when you know that your sample could make problems during the polishing process depending on the sample characteristics (stability, solubility, binding activity, etc.).

1. Select the right EndoTrap® system for your sample
   (here you have to try out <u>both</u> systems)
   1. **EndoTrap® blue**, using **your desired buffer** (enriched with 100 µM $CaCl_2$)
      or **EndoTrap® blue equilibration buffer**
      Equilibration buffer EndoTrap® blue: 20 mM Hepes, 150 mM NaCl, 0.1 mM $CaCl_2$, pH 7.5
   2. **EndoTrap® red**, using **your desired buffer**
      or **EndoTrap® red equilibration buffer**
      Equilibration buffer EndoTrap® red: 10 mM Na_2HPO_4/NaH_2PO_4, 80 mM NaCl, pH 7.4

<u>If you already know that PBS buffer is necessary:</u>

2. Select the right conditions (different PBS buffer compositions) for your desired application
   (here you can try out both systems; for EndoTrap® red you have to try out different buffers)
   1. **EndoTrap® blue**, PBS buffer (freshly enriched with 100 µM $CaCl_2$)
      10 mM Na_2HPO_4, 137 mM NaCl, 0.1 mM $CaCl_2$, pH 7.4
   2. **EndoTrap® red**, PBS buffer
      10 mM Na_2HPO_4, 137 mM NaCl, pH 7.4 (="classical PBS buffer")
   3. **EndoTrap® red**, PBS buffer (with only 80 mM NaCl, included in the EndoTrap® red kit)
      10 mM Na_2HPO_4/NaH_2PO_4, 80 mM NaCl, pH 7.4 (= Equilibration buffer EndoTrap® red)
   4. **EndoTrap® red**, PBS buffer (with only 20 mM NaCl, also available from Hyglos GmbH)
      10 mM Na_2HPO_4/NaH_2PO_4, 20 mM NaCl, pH 7.4 (= Low Salt Equilibration buffer)

If you prefer to get individual technical assistance from Hyglos, we kindly ask you to provide us with following information regarding your sample:

1. LPS contamination / concentration (EU/ml or EU/mg)
2. Bacteria strain
3. Substance that should be cleaned (e.g. name of protein, antibody)
4. Physical and/or chemical (e.g. LPS-binding protein) characteristics (of the sample)
5. Concentration (sample)
6. Volume (sample)
7. Detailed buffer composition (if you used your own buffer)
8. pH buffer (if you used your own buffer)
9. LAL assay (e.g. name, manufacturer)

Precautions

! EndoTrap® is supplied / stored with sodium azide, which is a poison. The toxicological properties of this product have not been fully investigated. At the low concentrations of sodium azide in this product no risk of poisoning is expected with normal use. Still avoid ingestion, inhalation and skin contact. For further information see the EndoTrap® Material Safety Data Sheet.

! All used materials like containers or pipette tips and buffers must be endotoxin free. Glass ware is preferred, as endotoxins can be removed by heat treatment (200°C, 4 h or 250°C, 1 h).

! Empty columns and funnels are delivered **not endotoxin free**. In order to exclude any co-contamination with LPS insert them in at least 1 M NaOH over night (6-12 h). Afterwards wash them with endotoxin free water and let them air dry. For details please inquire our protocol "Procedure for packing gel into a column".

! Buffers should be prepared from endotoxin free materials and endotoxin free water.

! Buffers, resin and sample should have the same temperature (4-20°C) during the cleaning steps.

! When using EndoTrap® columns, all buffers including equilibration buffer EB and regeneration buffer RB should be **degassed** prior to use. When using EndoTrap® gel slurry, degas slurry prior to use.

! EndoTrap® 10x buffers (Item. No. 800003 & 800004, also contained in EndoTrap® 50: Item No. 311075 & 321075) have to be diluted 1:10 with endotoxin-free water.

! Avoid proteases (see page 8) and organic solvents.

! **If you use your customer specific buffers and EndoTrap® blue:**
Buffers used for endotoxin removal with EndoTrap® blue need to **contain 50-100 µM Ca^{2+}**.

Definition: **column volume** = used settled EndoTrap® resin (e.g.: "ready-to-use" 1 ml column = 1 ml)
gel volume = used settled EndoTrap® resin (after removing storage buffer from gel slurry)

Protocols

Chromatography is traditionally done in two modes: discontinuous (batch mode) and continuous (column mode) chromatography. EndoTrap® can be used either in batch or column mode. In general endotoxin removal of high endotoxin levels is more practical in the column mode. Batch mode may be used for small volumes or to increase contact time. **However, parameters such as pH, ionic strength, temperature, contact time etc. might have to be optimized for each application to obtain maximum endotoxin removal with minimum loss of product.**

If you want to pack the columns (small / large plastic columns) by yourself we provide a protocol "Procedure for packing gel into a column".

We also provide a protocol for HPLC / FPLC automated systems ("Application Protocol for Pilot Scale") for customers who prefer using their liquid chromatography systems.
Please inquire for our special protocols at www.hyglos.de!

The standard protocol for column / batch mode is described in the package insert!

Protocol overview:

- Optional*: Dialysis of the antibody solution against Low Salt Equilibration buffer[1]
- Endotoxin removal with EndoTrap® red
- Optional*: Dialysis of the antibody solution against e.g. PBS[2]
- Detection of antibody and endotoxin concentrations

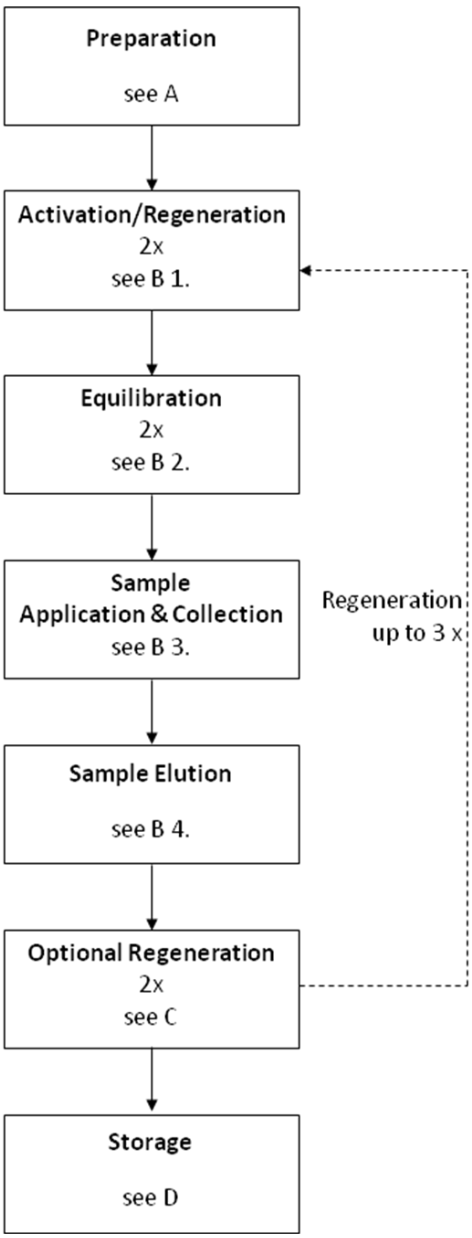
* “Dialysis protocol” see page 7

Detailed protocol: Endotoxin removal with EndoTrap® red

- Make a working solution of antibodies with a recommended end-concentration of 1-10 mg/ml (diluted in Low Salt Equilibration buffer).

A Preparation

- To use a prepacked column place the column in a suitable holder and remove the top cap first. This prevents air bubbles from being soaked up. Next remove bottom cap. Allow the storage solution to drain from the column. [~ 8 min] The flow stops automatically when the solution reaches the upper disc. Make sure to never let the EndoTrap® resin run dry!



B Activation of EndoTrap® column:

1 Fill up the column with **regeneration buffer** (RB) [this corresponds to ~3 ml or **3 column volumes**] and let the column drain out completely. Repeat this step. [~12 min]

2 Fill up the column with **equilibration buffer** (EB)[3] or customer specific buffer (here: Low Salt Equilibration buffer[4]) [this corresponds to ~3 ml or **3 column volumes**] and let the column drain out completely. Repeat this step. [~12 min]

3 Apply your **antibody solution** (either in **equilibration buffer** (EB) or in customer specific buffer) onto the column and start collecting your fractions (depending on the applied sample volume) into a sterile tube immediately. Applied sample elutes directly after the column void volume (0.3-0.5 ml). The column can be constantly filled up, until the whole sample (up to 50 ml) is completely filled in. Afterwards let the sample drain completely from column. [flow rate: 0.2-1 ml/min]

4 In order to elute your entire sample, apply extra **equilibration buffer** (EB) or customer specific buffer (e.g. 1 ml or 1 column volume), let the column drain out and collect the flow through completely. [As substances pass through the column at different rates, it is important to test each fraction for the sample concentration. This can be done for example by measuring the optical density of the flow through fractions.]

C Regeneration:

- Fill up the column with equilibration buffer (EB) or customer specific buffer [this corresponds to 3 ml or 3 column volumes] and let the column drain out completely. Repeat this step [~ 12 min]. Continue with step B1 of “Activation of EndoTrap® Column”.

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[1] **Low-Salt Equilibration buffer:** 10 mM Na_2HPO_4/NaH_2PO_4, 20 mM NaCl, pH 7.4, endotoxin-free

[2] **PBS-Buffer:** 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4, endotoxin-free

[3] **Equilibration buffer “red” (EB):** 10 mM Na_2HPO_4, 80 mM NaCl, pH 7.4, endotoxin-free

[4] **Low Salt Equilibration buffer:** 10 mM Na_2HPO_4/NaH_2PO_4, 20 mM NaCl, pH 7.4, endotoxin-free

D Storage:

- Fill up the column with **equilibration buffer** (EB) or customer specific buffer [this corresponds to 3 ml or 3 column volumes] and let the column drain out completely. Repeat this step. [~ 12 min]
- Fill up the column with 1 ml of EndoTrap® **regeneration buffer** (RB) supplemented with 0.02% sodium azide and store at 4 °C.

Please consider: **The used Low Salt Equilibration buffer (Article No. 321082) is not included in the EndoTrap® red kit but it can be ordered separately.**

Optional steps:

Detection of antibody and endotoxin concentrations

Endotoxin Detection (e.g. LAL assay):

5 Check for endotoxin removal using an appropriate assay

6 In case the LPS contamination is still to high make a second LPS removal step with EndoTrap®.

Optional finishing step:

7 Combine the fractions and filtrate the solution over 0.2 µm membranes (e.g. Pall Supor® [PES, Polyethersulfone]) to ensure sterility.

Antibody Recovery Rate:

8 Measure the antibody concentration with e.g. BCA™ Protein Assay from Pierce.

Dialysis of the antibody solution

1. Prepare an appropriate amount of dialysis buffer (e.g. PBS) using filtered water and sterile buffer solutions. Dialyses buffer and container should be endotoxin free.
2. Use appropriate dialysis membrane. The **MWCO (molecular weight cut off) should be 6 times below the molecular weight of the antibody.** *e.g.* for IgG the MWCO should be about 25000 or smaller.
3. Prepare the dialysis tube according to instructions of the manufacturer.
4. Seal the dialysis tube at one end with a dialysis clamp, fill the antibody into the dialysis tube, close the tube with a second clamp, and place the dialysis tube into a container filled with dialysis buffer. **The volume of the dialysis buffer should 100-1000 times larger than the volume in the dialysis tube.**
5. Stir the dialysis buffer at 4 ˚the dialysis buffer at 4s buffer should 100-1000 times larger than the volume in the dialysis tube.lose the tube with a second clamp, and
6. Remove the solution out of the dialysis tube and determine the concentration of the antibody.

Storage and Handling of Antibodies

The most usual problem of storing antibody solutions is microbial contamination and therefore also contamination with endotoxins. Contamination can be avoided by the addition of sodium azide (0.1%). It is recommended to add sodium azide for long term storage of sera. Serum containing antibodies should be stored at -20°C. Antibody solutions should not be frozen and thawed repeatedly as this can lead to the loss of activity. It is recommended that sera are stored in convenient working aliquots at -20°C. Antibodies should be stable for several years when stored at

this temperature. Consider: Serum that is sterile or stored at -70°C does not require the addition of preservatives.

Antibody solutions will often form an insoluble lipid component with prolonged storage. The lipid layer can be removed by centrifugation at 10,000 x g. If the lipids form a layer above the aqueous phase, remove the aqueous phase and store as suggested above. Consider: The formation of a lipid layer in antibody solutions containing sodium azide is not indicative of a microbial contamination.

Literature:

- Monoclonal Antibody Production
  A Report of the Committee on Methods of Producing Monoclonal Antibodies Institute for Laboratory Animal Research National Research Council.
  NATIONAL ACADEMY PRESS Washington, DC 1999

- Pharmaceutical Committee
  Production and Quality Control of Monoclonal Antibodies
  http://pharmacos.eudra.org/F2/eudralex/vol-3/pdfs-en/3ab4aen.pdf

- MONOCLONAL ANTIBODY INDEX©
  The **MONOCLONAL ANTIBODY INDEX** is a fully searchable biotechnology database with yearly updated information on the most important monoclonal antibodies produced for the diagnosis and therapy of human cancer, AIDS, SARS and other infections, heart , vascular and coagulation diseases, transplantation, inflammation, Alzheimer's disease, autoimmunity and other pathologies or uses, indicating all available data on:
  - Antibody name(s)
  - Antibody species
  - Antibody type
  - Antibody characteristics
  - Related antibodies
  - Target tissue/cell type
  - Target disease or use
  - Antigen name (with description table)
  - Antigen molecular weight
  - Antigen characteristics
  - Clinical study phase
  - Patent assignee, producer, developer or distributor and their respective technical or commercial names for the antibody, antigen or derivatives.
  - References of production, characteristics and use obtained from journals, patents, abstracts, press releases and reports available until January 2005.

  http://www.gallartinternet.com/mai/

- 2006 Cambridge Antibody Technology
  Antibody Glossary
  http://www.cambridgeantibody.com/html/glossary

Workflow: EndoTrap® for endotoxin removal and subsequent endotoxin detection with EndoLISA & EndoZyme

As a complete workflow solution Hyglos recommends the combination of EndoTrap® and EndoLISA . This system is the perfect combination for a workflow including an endotoxin removal and an endotoxin detection step. Please see www.EndoLISA.com & www.EndoZyme.com for further details.

Hyglos Services: endotoxin removal

Hyglos offers an endotoxin removal service.

Use our expertise for your product: Endotoxin removal service!

With this service best results were already achieved with even the most challenging products like tricky to handle antibodies and plant extracts.

Our **endotoxin removal service** includes:

- Removal of endotoxin with the adapted protocol
- Endotoxin detection before and after the removal steps as described above in the "endotoxin detection service"

Please inquire for our services.

For inquiries and technical support please contact:

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If you like to learn more about our products and services, please visit our website www.hyglos.de