Package Insert EndoTrap® red

- **Cat. No. LET0001 - EndoTrap® red 1/1**
- **Cat. No. LET0002 - EndoTrap® red 5/1**
- **Cat. No. LET0033 - EndoTrap® red 5**
- **Cat. No. LET0003 - EndoTrap® red 10**
- **Cat. No. LET0004 - EndoTrap® red 50**
- **Cat. No. LET0032 - EndoTrap® red Buffer Kit**

For laboratory and research use only. Not for use in diagnostic procedures.

Store the kits at +2 to 8 °C

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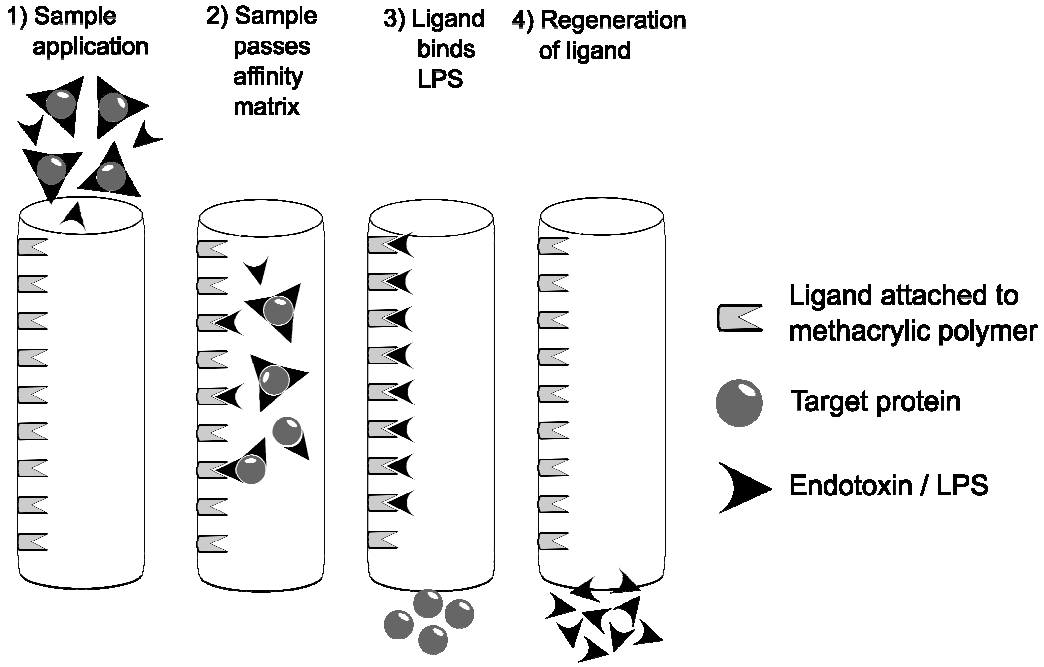
1. General Information

1.1 Intended Use

EndoTrap® is an affinity matrix intended for removal of lipopolysaccharide (LPS) from biological samples in aqueous solutions such as proteins, antibodies, cell extracts and nucleic acids. EndoTrap® can be employed in both batch and column mode.

1.2 Principle



1.3 EndoTrap® red Kit Components

Kit Components	
EndoTrap® red 1/1	**EndoTrap® red 5/1**
1x1 ml EndoTrap® red column	5x1 ml EndoTrap® red column
25 ml EndoTrap® red 5x Equilibration buffer	125 ml EndoTrap® red 5x Equilibration buffer
25 ml EndoTrap® red 5x Regeneration buffer	125 ml EndoTrap® red 5x Regeneration buffer

EndoTrap® red Buffer Kit
125 mL EndoTrap® red 5x Equilibration Buffer
125 mL EndoTrap® red 5x Regeneration Buffer

Single components		
EndoTrap® red 5	**EndoTrap® red 10**	**EndoTrap® red 50**
5 ml settled resin	10 ml settled resin	50 ml settled resin

1.4 Specifications

EndoTrap® ligand	LPS-specific bacteriophage derived protein
Binding capacity	2 x 10^6 EU/ml resin (1 EU = 100 pg LPS)
Dissociation constant	K_D = 5 x 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 to 1 ml/min
Max. pressure	**0.3 MPa** (when using automated systems), 43 psi
Temperature stability	2 – 35°C
Storage	At 2 - 8°C in regeneration buffer supplemented with 0.02% sodium azide. **Do not freeze!**
Shelf life	EndoTrap® is stable until the stated expiry date when stored according to instructions.

1.5 Precautions

- EndoTrap® 5x buffers (Cat. No. LET0032 also contained in EndoTrap® red 1/1 and 5/1 (Cat. No. LET0001 and LET0002) have to be diluted 1:5 with endotoxin-free water prior to use.
- Buffers, resin and samples should have the same temperature (4 - 20°C) during the cleaning steps.
- Buffers must be prepared from endotoxin-free materials and endotoxin-free water.
- All materials used, such as containers, pipette tips and buffers, must be endotoxin-free. Glass ware is preferred, as endotoxins can be destroyed by heat treatment (200 °C, 4 h or 250 °C, 1 h). Endotoxin-free EndoGrade® Glass Test Tubes (capacity 5 ml) are supplied by LIONEX.
- Empty columns and funnels are available from LIONEX and supplied **not endotoxin-free**. In order to exclude any co-contamination with LPS, empty columns and funnels should be inserted in at least 1 M NaOH overnight (6 - 12 h), subsequently washed with endotoxin-free water and air dried. The protocol “Procedure for packing gel into a column” is available from LIONEX on request.
- EndoTrap® resins and pre-filled columns are supplied with ProClin® as a preservative. For safety information see the EndoTrap® Material Safety Data Sheet.
- Proteases may destroy the EndoTrap® ligand during LPS removal. Please perform the cleaning steps at conditions where the protease is less active, e.g. 4°C, or change the buffer composition if possible.

The most important considerations for EndoTrap® red:

	EndoTrap® red
▪ pH (buffer)	pH 6-9
▪ Highest ionic strength possible	250 mM NaCl ***best results at < 100 mM NaCl***
▪ Suitable with EDTA and other calcium chelators containing buffers	Yes
▪ Customer specific equilibration buffer has to be enriched with calcium	No
▪ PBS can be used as equilibration buffer *See 3.3 on page 11 for further details*	Yes, “half-concentrated” PBS should be used

1.6 Storage

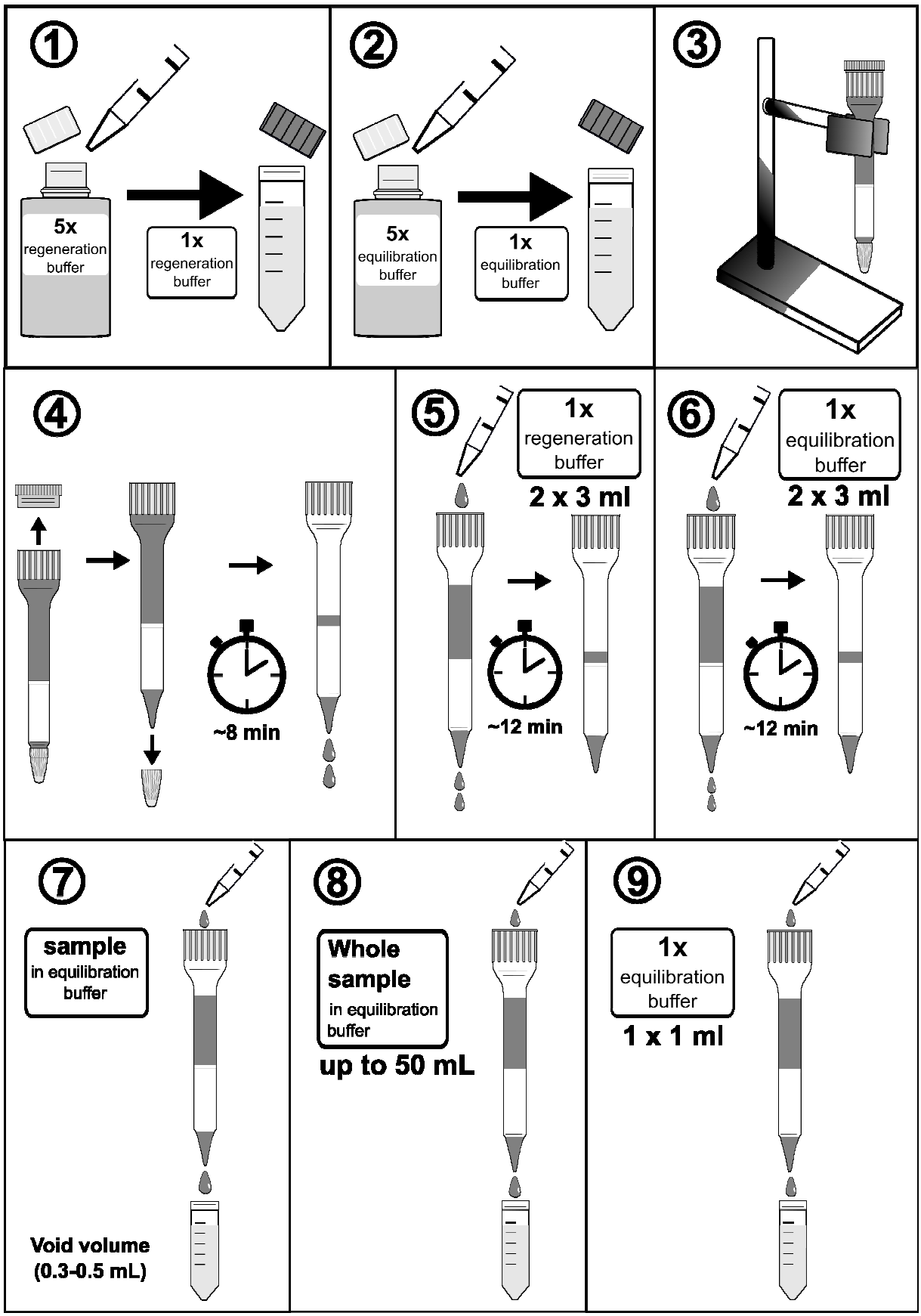
EndoTrap® red resin and buffers should be stored at 2-8°C. After using the regenerated resin should be stored in regeneration buffer supplement with **2.5 ppm ProClin™ or 0.02% sodium azide** (shelf life until the indicated expiry date). Alternatively, 20% ethanol can be used as storage buffer; the storage time will then be reduced to 4 weeks.

1.7 Short protocol – Column mode

Notes before starting

- A sample volume of 2 - 10 ml is recommended (the maximum sample volume is 50 mL).
- The sample concentration shall not exceed 1 mg per mL resin.
- To avoid the dilution of the sample smaller fractions can be collected and tested by protein assay.

1. Dilute 5x regeneration buffer with endotoxin-free water.
2. Dilute 5x equilibration buffer with endotoxin-free water.
3. Place column in a suitable holder.
4. First remove the top cap and then the bottom cap of the prepacked column. Allow the storage solution to drain from the column.
5. Fill the column with 3 mL regeneration buffer and let the column drain out completely. Repeat this step
6. Fill the column with 3 mL **equilibration buffer** or customer specific buffer and let the column drain out completely. Repeat this step.
7. Apply your **sample** (either **in equilibration buffer** or in customer specific buffer) onto the column and let drain off the void volume of 0.3-0.5 mL.
8. Directly after the void volume the sample elutes and can be saved. A sample volume up to 50 mL can be applied.
9. To elute the entire sample the column can be rinsed with 1 mL equilibration buffer.


①
5x
regeneration
buffer
1x
regeneration
buffer
②
5x
equilibration
buffer
1x
equilibration
buffer
③
④
~8 min
⑤
1x
regeneration
buffer
2 x 3 ml
~12 min
⑥
1x
equilibration
buffer
2 x 3 ml
~12 min
⑦
sample
in equilibration
buffer
Void volume
(0.3-0.5 mL)
⑧
Whole
sample
in equilibration
buffer
up to 50 mL
⑨
1x
equilibration
buffer
1 x 1 ml

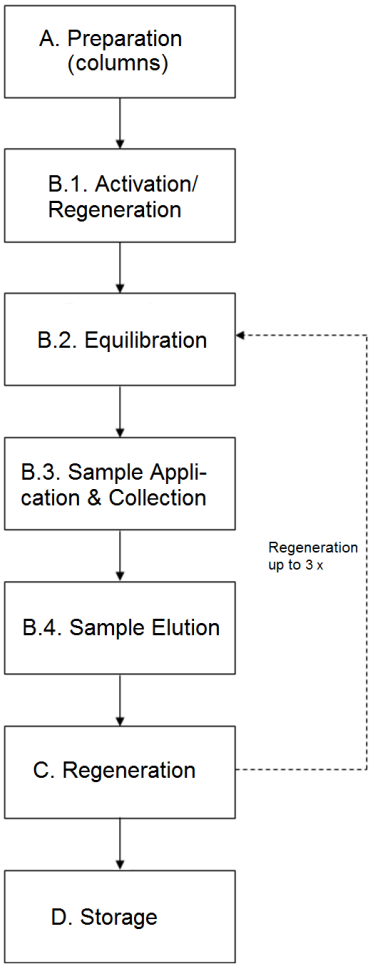
2. EndoTrap® Protocols

Chromatography is commonly performed in two modes: continuous (column mode) chromatography and discontinuous (batch mode).
EndoTrap® can be used in both column and batch mode. Removal of high endotoxin levels is generally more convenient in the column mode. Batch mode may be used for small volumes or to increase contact time. Parameters such as pH, ionic strength, temperature and contact time should be optimized for each application to obtain maximum endotoxin removal with minimum product loss.

2.1 Protocol Column Mode

A. Preparation

To use a **prepacked column** place the column in a suitable holder and first remove the **top cap**. This prevents air bubbles from emerging. 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 dry out! **5x buffers must be diluted 1:5 with endotoxin-free water prior to use**.



B. Activation and Endotoxin Removal

1. Fill the column with 3 ml **regeneration buffer**[1] and let the column drain off completely. Repeat once [~12 min].

2. Fill the column with 3 ml **equilibration buffer**[2] or customer specific buffer and let the column drain off completely. Repeat once [~12 min].

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

4. In order to elute the entire sample, apply additional 1 ml **equilibration buffer** or customer specific buffer, let the column drain off 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 by for example measuring the optical density of the flow through fractions.]

C. Regeneration

Fill the column with 3 ml **regeneration buffer** or customer specific buffer and let the column drain off completely. Repeat once [~ 12 min]. **Continue with step B.2**.

D. Storage

Apply 1 ml regeneration buffer, **supplement with** ProClin® as **preservative** and let the column drain off completely. Close the bottom cap of the column and apply 1 ml regeneration buffer, **supplement with** ProClin® and store at 2 to 8°C (shelf life until the indicated expiry date). Alternatively 20% ethanol can be used as storage buffer; the storage time will then be reduced to 4 weeks.

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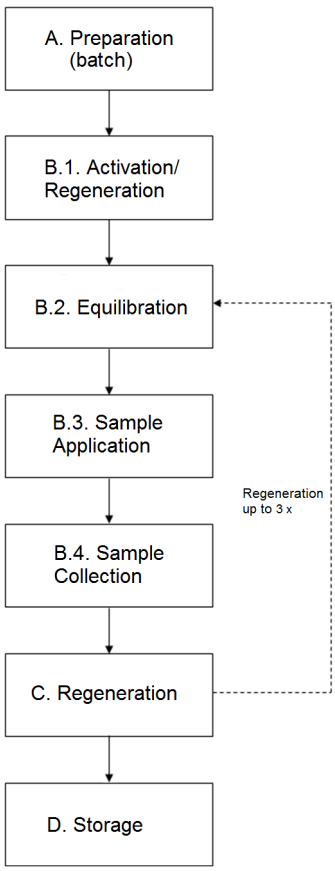
1 The regeneration substance is NOT (sodium) deoxycholate! DOC would have cytotoxical effects on cell culture and also influence the cell growth and the morphology of the cells. It is reported that DOC induces DNA damage.
2 5 x Equilibration buffer “red”: provided in the kit (only LET0001, LET0002)

2.2 Protocol Batch Mode

A. Preparation

A ratio of 2:1 to 10:1 between sample and resin volume is recommended (up to 50 ml sample per ml resin is possible). All centrifugation steps should be carried out at ~ 1200 x g for 5 min (bench top centrifuge)! Several contact times should be tested to determine the optimal contact time for endotoxin removal. Remove the storage buffer from the gel slurry by centrifugation and discard the supernatant. 10x buffers have to be diluted 1:10 with endotoxin-free water prior to use.



B. Activation and Endotoxin Removal

1. Add 2 gel volumes of **regeneration buffer**[1], mix by gently shaking the tube for 5 sec; centrifuge, and discard the supernatant. Repeat twice.

2. Add 2 gel volumes of **equilibration buffer**[2] or customer specific buffer, mix by gently shaking the tube for 5 sec; centrifuge and discard the supernatant. Repeat twice.

3. Add the sample (either in **equilibration buffer** or in customer specific buffer) and incubate for at least 5 min. Gently shake or rotate the tube while incubating.

4. Centrifuge at ~ 1200 x g for 2 min (bench top centrifuge) and transfer the supernatant (sample) to an endotoxin-free tube.

C. Regeneration

Resuspend the EndoTrap® gel pellet in 2 gel volumes of **regeneration buffer**, mix by gently shaking the tube for 5 sec; centrifuge and discard the supernatant. Repeat twice. Continue with **step B2**.

D. Storage

Resuspend the EndoTrap® gel pellet in 1 gel volume of regeneration buffer (RB), **supplemented with ProClin®** and store at 2 to 8°C (shelf life until the indicated expiry date).
Alternatively 20% ethanol can be used as storage buffer; the storage time will then be reduced to 4 weeks.

2.3 How to work with a real flow through system

EndoTrap® is a **real flow through system** (“ready-to-use” columns). Apply the sample (up to 50 ml) continuously to a 1 ml column – up to 3 ml at once. With an additional funnel, 20 to 25 ml can be filled at once.

2.4 Optional Steps (Column / Batch Mode)

Endotoxin / LPS detection:
- Check the LPS removal efficiency using an endotoxin detection assay. If the LPS contamination is still too high, perform a second LPS removal step.

Protein polishing / recovery:
- Combine the fractions and filtrate the solution over 0.2 µm membranes to ensure sterile conditions.
- Measure the protein concentration with appropriate methods or measure the absorption at 280 nm.

[1] The regeneration substance is NOT (sodium) deoxycholate! DOC would have cytotoxical effects on cell culture and also influence the cell growth and the morphology of the cells. It is reported that DOC induces DNA damage.
[2] 5 x Equilibration buffer “red” (EB): provided in the kit (only LET0001, LET0002)

3. Additional Information

3.1 How to calculate the number of required cleaning steps

EndoTrap® red can be re-used at least three times (EndoTrap® HD at least 10 times) without any loss of endotoxin removal efficiency. If the initial endotoxin concentration is very high or if a very low concentration has to be reached, EndoTrap® can be applied several times in a consecutive manner. Each round of application theoretically yields a two log reduction of endotoxin.

Parameters such as pH, ionic strength, temperature, contact time, etc. can be optimized for each application to obtain maximum endotoxin removal with minimum loss of product.

Depending on the LPS starting concentration [EU] (1 EU = 100 pg LPS), perform a certain number of cleaning steps, in order to achieve the desired LPS end concentration [EU]. To achieve best results, total LPS units applied should not exceed 30 to 50% of the maximum resin capacity.

Starting LPS concentration (buffer) [EU]	After 1. cleaning step [EU]	**regeneration step** (kit includes regeneration buffer)	After 2. cleaning step [EU]	**regeneration step** (kit includes regeneration buffer)	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®, concentrations lower than 0.005 EU/ml can be achieved.

LPS removal from proteins:
With repetitive use of EndoTrap®, concentrations lower than 0.1 EU/ml can be achieved. The efficiency of EndoTrap® slightly decreases at low endotoxin contamination levels (0.1 EU/ml), at this concentration the removal efficiency is approximately 70%.

Note that each cleaning step ends is followed by resin (ligand) regeneration.

3.2 Custom Specific Buffers for EndoTrap® red

Custom specific buffers can be used for equilibration and endotoxin binding.

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)

3.3 Type of Samples which can be used with EndoTrap® red

	EndoTrap® red
Tested substances which can be applied onto the column	▪ proteins ▪ peptides ▪ antibodies
Regeneration buffer (endotoxin concentration < 0.05 EU/ml)	Regeneration buffer "red" (based on "**Phosphate buffer**", pH 7.4)
Equilibration buffer (endotoxin concentration < 0.05 EU/ml)	Equilibration buffer "red" ("**Phosphate buffer**", pH 7.4 **with 80 mM NaCl**)
Other suitable equilibration buffers	**PBS, HEPES, Borate, TRIS, MOPS, MES, PIPES, Citrate, Acetate, Glycine** and **Carbonate buffers**
pI of applied proteins	pI from 5 - 9
pH (buffer)	pH 6 - 9
Ionic strength	up to 250 mM NaCl, ***< 100 mM NaCl is recommended***
Recommended working concentration of applied substances	1 - 10 mg/ml
Recommended sample volume per ml resin	up to 50 ml
Tested substances which **do not interfere** with the performance of EndoTrap® red	DTT not tested max. 0.005% NaDOC 20% DMSO 20% Isopropanol 40% Methanol 20% Ethanol 20% Glycerol / Glycerin 1 M Urea 300 mM Imidazole
Tested substances which may **interfere** with the performance of EndoTrap® red and therefore having an **inhibitory effect** on LPS binding	▪ > 250 mM NaCl ▪ SDS, Tween20 and other detergents ▪ GdnHCl ▪ Ammoniumsulphate
Tested LPS-types (bacterial strain)	▪ *Escherichia coli* K12 ▪ *Salmonella enterica* ▪ *Citrobacter freundii* ▪ *Pseudomonas aeruginosa* **Use EndoTrap® red for:** ▪ ***Klebsiella pneumoniae*** ▪ ***Serratia marcescens***

3.4 Trouble Shooting Guide

Please consider the chemical characteristics of the used sample before choosing one improvement step.

Issue	**EndoTrap® red**
… low sample recovery rate …	
- due to **ionic interactions**	EndoTrap® red is not suitable for high ionic strength – please use EndoTrap® HD.
- due to **interactions** with **lipopolysaccharides**	Hydrophobic interaction of samples with LPS may occur. As lipopolysaccharides form aggregates, it might also be possible that your sample arranges within these aggregates. It may help to disintegrate the aggregates or to reduce their size. For that purpose Triethylamine (combined with 15 min ultrasonic treatment) or detergents can be used. EndoTrap® is suitable for buffers containing 20% glycerol. Note: Detergents may interfere with endotoxin detection in the LAL assay. In this case.
- due to **negative charge** of the sample	EndoTrap® red ligand **could** interact with negative charge – use EndoTrap® HD instead.
- due to **interactions with calcium**	EndoTrap® red does not need calcium ions

… low LPS removal rate …	
- due to **depletion of calcium**	EndoTrap® red does not need calcium ions
- due to interference with **buffer additives**	Add up to 40% ethanol to the equilibration buffer or customer specific buffer.
- due to limiting **contact time**	1. Column mode: Use half of the sample or use a smaller column, alternatively close column bottom cap to achieve a longer contact time (max. overnight). 2. Batch mode: Increase the contact time or the resin to sample ratio.
- due to limiting **LPS binding capacity**	To achieve best results, total LPS units applied should **not exceed 30 to 50%** of the maximum column capacity (2 x 10^6 EU/ml resin).
… slow flow through rate (<< 0.2 to 1 ml/min [gravity flow]) …	
- due to **viscous solutions**	EndoTrap® red is **not** suitable for viscous solutions! For viscous solutions EndoTrap® HD (www.lionex.de) is recommended in batch mode.
- due to **bubbles**	Remove bubbles by centrifuging the closed column (filled with buffer by a height of 1 - 2 cm) at ~ 1000 x g for 5 min. (using a “clinical-type” centrifuge, i.e. one with swinging baskets works best). For this procedure please place the column into a suitable 50 ml tube.

3.5 Related products

EndoTrap® HD*	Description	Cat. No.
EndoTrap® HD 1/ 1	1 x 1 mL EndoTrap® HD columns 25 mL 5x EndoTrap® HD Equilibration Buffer 25 mL 5x EndoTrap® HD Regeneration Buffer 25 mL 5x EndoTrap® HD Storage Buffer	LET0009
EndoTrap® HD 5/ 1	5 x 1 mL EndoTrap® HD column 125 mL 5x EndoTrap® HD Equilibration Buffer 125 mL 5x EndoTrap® HD Regeneration Buffer 125 mL 5x EndoTrap® HD Storage Buffer	LET0010
EndoTrap® HD column	1 ml column	LET0035
EndoTrap® HD 5	5 mL settled resin (50% slurry)	LET0023
EndoTrap® HD 10	10 mL settled resin (50% slurry)	LET0011
EndoTrap® HD 50	50 mL settled resin (50% slurry)	LET0012
EndoTrap® HD 250	250 mL settled resin (50 % slurry)	LET0013

* EndoTrap® HD offers best-in-class endotoxin binding capacity of **> 5,000,000 EU/ml** and can be **reused more than 10 times**. Moreover, EndoTrap® HD has been especially optimized for application in biomanufacturing processes. It can be used in early or late bio-manufacturing process steps. EndoTrap® HD is based on a hydrophilic, dimensionally stable affinity matrix with excellent pressure/flow characteristics. A Regulatory Support File can be provided on request. EndoTrap® HD is available as pre-filled columns (1x 1 ml and 5x 1 ml, including all necessary buffers) and settled resins (5 mL, 10 ml, 50 ml, 250 ml and bulk).

Endo Grade® Water	Description	Cat. No.
Endo Grade® Water, 50 mL	1 bottle	LET0036
Endo Grade® Water, 100 mL	1 bottle	LET0037
Endo Grade® Water, 500 mL	1 bottle	LET0038

Other products		
Disposable Polypropylene Funnel	(pack of 5)	LET0018
Empty Columns Large	(pack of 5)	LET0019
Empty Columns Small	(pack of 5)	LET0020
Endo Grade® Glass Test Tubes	112 endotoxin-free borosilicate glass test tubes with aluminium screw cap, capacity 5 mL, height 50 mm	LET0021

4. Technical Support and Further Product Information

4.1 Inquiries and Technical Support

Internet

Visit EndoTrap® on LIONEX' website **www.lionex.de** for:

Technical resources including manuals, application notes, Certificates of Analysis, Material Safety Data Sheets (MSDS), FAQs, customer publications and reference customers
Complete technical service contact information
Access to price lists and ordering forms
Additional product information and special offers

Contact us

For more information or technical assistance, call, write, fax or e-mail.

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4.2 Legal Statements and Patent Information

Trademarks

EndoTrap® and EndoGrade® are registered trademarks exclusively licensed to LIONEX GmbH
Tween20® is a registered trademark of ICI America, Inc.

Patent information

Parts of EndoTrap® products are protected under the following patents: EP1516188 and EP1695085

4.3 Related Products by LIONEX

EndoTrap® HD

- **EndoTrap® HD** Endotoxin removal system for High-Definition sample purification

EndoTrap® HD Leakage ELISA

- **EndoTrap® HD Leakage ELISA** for determination of EndoTrap® HD binding ligand

EndoGrade® Endotoxin-free Accessories

- **EndoGrade® Glass Test Tubes** - Endotoxin-free borosilicate glass test tubes with screw cap

EndoGrade® Endotoxin-free Reagents

- **EndoGrade® Ovalbumin** - Ultra-pure Ovalbumin for advanced immunology research

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