

Protocol for endotoxin-free plasmid DNA with JETSTAR 2.0 Maxi (September 2005)

IMPORTANT NOTE: This protocol is a modification of the JETSTAR 2.0 standard protocol to remove even last traces of bacterial endotoxin from plasmid DNA preps from *E. coli* cultures. **Please refer also to the standard protocol as all comments and recommendations for the standard procedure will pertain to the ,endotoxin-free procedure‘ as well.**

When working with gram-negative bacteria in the lab, endotoxins are likely to be present on nearly all parts that are in use by multiple researchers (e.g. many parts of glassware). Endotoxin molecules are very resistant against physical means of inactivation (e.g. heat). Therefore even single-use items (e.g. plasticware disposables) should be considered to be contaminated with endotoxins if not expressively otherwise stated.

In order to maintain eluted DNA endotoxin-free, one has to be very careful not to „re-contaminate“ plasmid DNA again by endotoxin-contaminated water, chemicals, glass or plasticware.

Before starting the procedure please check the following points:

- a) If using glassware, „bake“ the parts for at least 6 hours at $\geq 200^{\circ}\text{C}$ in an oven. Autoclaving does NOT destroy endotoxins – as an autoclave is normally used to decontaminate bacterial cultures, it can even be a massive source for endotoxins.
- b) Use only plasticware that is certified to be endotoxin- or pyrogen-free by the respective vendor.
- c) If preparing buffers and solutions yourself, all chemicals should be taken from fresh unopened bottles. The set of reagents used to prepare endotoxin-free buffers should be marked accordingly to keep the chemicals endotoxin-free.
- d) Endotoxin-free water to prepare 70% ethanol is supplied with each kit. Add the appropriate amount of absolute (96-100%) ethanol as stated on the bottle's label and mix thoroughly.

Protocol:

- 1.) Inoculate a plasmid-containing *E. coli* culture according to good microbiological practice in growth medium with the appropriate antibiotic and grow the cells for 16 – 18 h (usually overnight) or even 24 – 36 h if using a buffered growth medium.



- 2.) Equilibrate the column with **30 ml buffer E4**. Apply the buffer to the column and let it flow through until all liquid has drained from the column. Do not force out remaining liquid!



- 3.) For a Maxi prep with a high-copy plasmid 100 ml of *E. coli* culture should be processed. Spin down the cells for **3 min at $\geq 12,000 \times g$** . Remove the supernatant completely with a pipet.



- 4.) Resuspend the cells completely in **10 ml buffer E1 reconstituted with 100 $\mu\text{g/ml}$ RNase (provided with the kit)**. No cell clumps must be visible.



- 5.) Lyse the bacterial cells with **10 ml buffer E2**. Mix gently but **thoroughly** until a **homogeneous mixture** is obtained. Due to the release of genomic DNA the mixture is very viscous at this stage. Incubate at **room temperature** for **5 min**.



- 6.) Neutralize the lysis mix from step 5 with **10 ml buffer E3**. Mix gently but **thoroughly** until a **homogeneous mixture** is obtained. The liquid of the neutralized lysate must be **completely non-viscous again**. NO remainders of the viscous matter obtained during step 5 must be left!!



- 7.) Centrifuge the lysate for **at least 10 min** at **$\geq 12,000 \times g$** at **room temperature**. Transfer the clear supernatant particle-free into a fresh tube.



- 8.) Mix the supernatant with **1/10 volume of buffer ENDO-1**. For example add 3 ml of buffer ENDO-1 to 30 ml cleared lysate and mix well by inverting. **It is of utmost importance, that cleared lysate and buffer ENDO-1 are mixed to homogeneity.**



- 9.) Apply the cleared lysate/ENDO-1 mixture from step 8 onto the previously equilibrated (*step 2*) JETSTAR 2.0 Maxi column. Let all lysate run through the column by gravity flow until the column stops dripping. **DO NOT FORCE OUT REMAINING LIQUID!**



- 10.) Carry out a first wash of the JETSTAR 2.0 Maxi column with **30 ml buffer ENDO-2**. Let all buffer ENDO-2 run through the column by gravity flow until the column stops dripping. **DO NOT FORCE OUT REMAINING LIQUID!**



- 11.) Carry out a second wash of the JETSTAR 2.0 Maxi column with **30 ml standard wash buffer E5**. Let all buffer E5 run through the column by gravity flow until the column stops dripping. **DO NOT FORCE OUT REMAINING LIQUID!**



- 12.) Elute the DNA from the JETSTAR 2.0 Maxi column into a fresh endotoxin-free tube with **15 ml buffer E6**. Let all buffer E6 run through the column by gravity flow until the column stops dripping. **DO NOT FORCE OUT REMAINING LIQUID!**
Precipitate the DNA from the eluate with **0.7 vol isopropanol** and spin it down for at least 30 min at $\geq 12,000 \times g$ in a chilled (4°C) centrifuge.



- 13.) Discard the supernatant, wash the DNA pellet with 5 ml endotoxin-free 70% ethanol and re-centrifuge as described in step 12 for 5-10 min. Discard the supernatant and dry the DNA pellet.

This can be carried out at elevated temperature (e.g. 10-15 min at 65-70 °C) or under vacuum. **Be careful when using vacuum to not overdry the DNA, as overdried DNA will not dissolve readily.**

Finally dissolve the DNA pellet in a suitable volume of endotoxin-free TE buffer (supplied with the kit). Alternatively, endotoxin-free 10 mM Tris [pH 8-9] or endotoxin-free water can be used as well.

JETSTAR 2.0 ,Endotoxin-free' Buffer Compositions:

Buffer E1: 50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 100 µg/ml RNase A

Buffer E2: 200 mM NaOH, 1%(w/v) SDS

Buffer E3: 3.1 M potassium acetate (pH 5.5 with acetic acid)

Buffer ENDO-1: Proprietary formulation

Buffer E4: 100 mM sodium acetate (pH 5.0 with acetic acid), 600 mM NaCl, 0.15% Triton X-100

Buffer ENDO-2: Proprietary formulation

Buffer E5: 100 mM sodium acetate (pH 5.0 with acetic acid), 800 mM NaCl

Buffer E6: 100 mM sodium acetate (pH 5.0 with acetic acid), 1.500 mM NaCl

TE buffer: 10 mM Tris-HCl (pH 8.0-8.5), 0.1 mM EDTA