Detoxification of EtBr

User manual

bondEX EtBr

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MACHEREY-NAGEL

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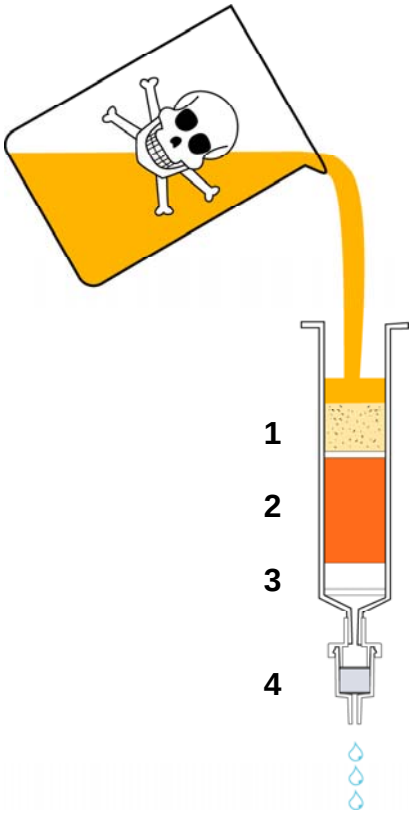
2 Product description

The most convenient way to visualize DNA in agarose gels is by staining with the fluorescent dye ethidium bromide (EtBr). Such substances intercalate between the bases of nucleic acids resulting in a higher fluorescence in comparison to the dye in free solution. Therefore, even small amounts of DNA can be detected. The disadvantage of EtBr-staining is the dye itself - EtBr and similar intercalating agents are powerful mutagens and toxic compounds, too. The decontamination and removal of waste is often a time consuming and expensive procedure.

2.1 The basic principle

In order to save time and money **MACHEREY-NAGEL** has developed the **bondEX EtBr** cartridges for an easy decontamination of intercalating dye-containing solutions. After equilibration of the cartridge with methanol or ethanol, the EtBr containing solution is simply poured onto the cartridge, which runs by gravity flow. To improve handling, the EtBr containing solution can be provided by a 1 l funnel or a 1-10 l tank to the bondEX EtBr cartridge via a connector. The mutagenic dye binds to the bondEX EtBr cartridge resulting in a flow-through free of EtBr. The binding efficiency is checked by a capacity indicator cartridge connected to the outlet of the bondEX EtBr cartridge.



The bondEX system includes:

- 1: prefiltration element
- 2: EtBr binding zone
- 3: white, dye-binding zone
- 4: removable indicator cartridge

2.2 Specifications of the bondEX EtBr system

- Up to **50 mg** of EtBr can be adsorbed by the **bondEX EtBr 50** cartridges.
- The **bondEX EtBr** cartridge is recommended for the extraction of EtBr or SYBR Green from diluted TAE or TBE gel buffers.
- **A maximum volume of 50 l filtered solutions containing up to 50 mg EtBr** can be decontaminated before flow rates decrease and cartridges are exhausted.
- **Prefilter element** for improved long term flow rate
- Removable capacity indicator cartridges for monitoring the bondEX EtBr capacity
- **Variable connectors** for a 1 l funnel or a 5-10 l tank

2.3 Important remarks and hints

1 EtBr solutions **> 1 mg/l** or high salt buffers should be diluted 1 : 4 with water before applying them onto the bondEX EtBr cartridge.

2 Solutions must not contain any organic solvents.

3 In general, 10 µl or less of a 10 mg/ml EtBr solution is used for a 100 ml agarose gel. In consequence, 1 l of gel buffer or staining solution usually contains about 0.1-0.5 mg/l EtBr depending on the number of gels used with this buffer. We recommend to

- calculate or at least estimate the EtBr content of the solutions
- collect the volume corresponding to the permitted maximal amount of EtBr e. g. 10-50 l and decontaminate it continuously.

4 Solutions containing particles or precipitates are not suitable without pretreatment. In order to prevent clogging of the cartridge, especially for buffers containing agarose particles, a **filtration step using our bondEX folded filters XL (supplied) has to be performed before decontamination**.

The bondEX system was developed for standard TBE and TAE gel buffers. Old or viscous e. g. staining solutions with precipitates / bacterial growth and unknown contents are not suitable. Filtration is essential for all solutions – "old" buffers, which are decontaminated by bondEX EtBr cartridges, may cause reduced flow rates in comparison with fresh solutions and a limited volume-capacity.

5 Faster flow-through rates as mentioned may lead to EtBr breakthrough! Do not use additional force! When using **new cartridges apply only half volumes** (500 ml on a 1 l funnel) or **regulate flow rates by clamps** (tank) until the optimal flow-through rate has been adjusted (about 10-20 ml/min).

6 Depending on the individual buffers (age, viscosity), flow rates decrease during use. Gel buffers should be decontaminated optimally soon after use.

7 Following our protocol more than 99% of EtBr is removed.

8 Be careful when working with EtBr solutions and decontaminated filtrate until absence of EtBr was verified. Follow EtBr safety data sheet instructions given by the supplier and the usual guidelines for toxic samples.

9 According to our best knowledge no uniform regulations exist regarding maximum concentration of EtBr allowed for disposal. Contact your local authorities for further information and regulations.

10 Ames and other tests (Microtox, Mutatox) indicating mutagenic or toxic effects of EtBr detect EtBr at concentrations about 50 µg/l. bondEX decontaminated filtrates show no toxic or mutagenic effects in the latter tests. No detailed studies are known about minimal concentration of EtBr sufficient for short or long term toxic or mutagenic effects.

The following **safety precautions** should be taken when using bondEX EtBr:

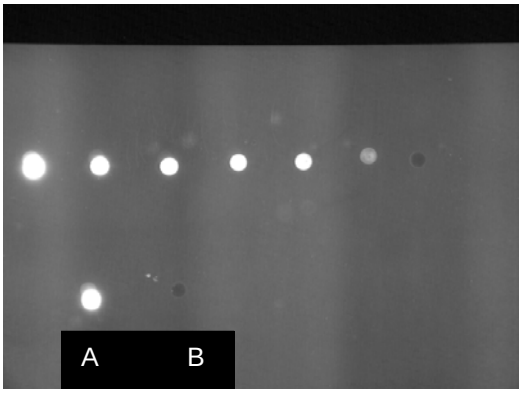
- Note the applied amount of EtBr solution to calculate end of capacity.
- Control the decontaminated solution by UV transillumination (saran wrap method) DC or UV spectroscopy 260 - 400 nm (also see section 3).
- Check the capacity status of the system with indicator cartridges.
- Follow guidelines for working with toxic and mutagenic substances given by local authorities and the ethidium bromide manufacturer.

3 Easy methods for checking EtBr concentrations and capacity

In order to determine the concentration of EtBr in TAE/TBE-buffer solutions or decontaminated filtrates we recommend four methods:

3.1 Saran wrap method

Mix 10-20 µl of a DNA containing solution (e. g. 0.5-2 µg/µl plasmid or calf thymus DNA in 1xTE or TAE buffer) with the equal volume of a series of EtBr concentration standards. Spot the mixtures in an ordered array on a sheet of Saran wrap (parafilm) stretched over an UV transilluminator. Under UV illumination an EtBr concentration of about 0.05-5 µg/ml is detectable by its fluorescence. The same test is done with the decontaminated solution. The EtBr fluorescence can be photographed for a better estimation of EtBr concentrations in comparison to the standard series. An exact determination is possible if the described assay is measured in a fluorescence spectrophotometer using 520/540 nm for excitation and 586nm for detection.

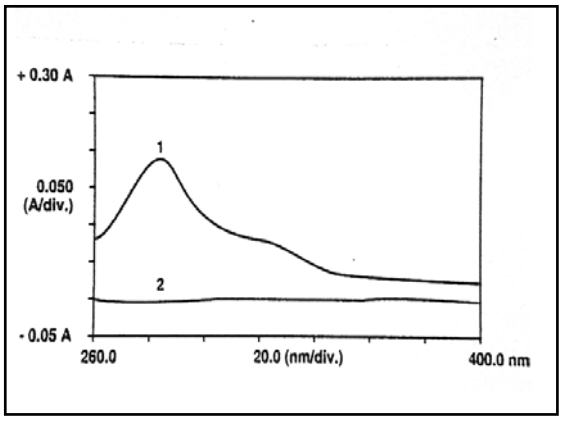


The picture shows a saran wrap with spots of decreasing concentrations of EtBr on a UV transilluminator. On the upper lane 5, 1, 0.5, 0.25, 0.1, 0.05 and 0 µg EtBr/ml TAE solutions were mixed with equal volumes of pBS in TE (15 µl each). Below a 2 µg/ml EtBr containing sample (A, left spot) was decontaminated – the BondEx flow-through contains no detectable EtBr (B, right spot).

3.2 TLC plate method

Pipette 5 µl solution onto a TLC plate (e. g. usual silica thin layer plates). EtBr containing solutions can be detected under an UV hand lamp by its fluorescence.

3.3 Spectrophotometric measurement



Measure a UV-spectrum between 260 and 400 nm and compare the solutions which contain EtBr and which are BondEx decontaminated. The absorption at about 285 nm indicating EtBr is absent in decontaminated solutions. Don´t forget to zero the background using the same buffer as blank. About 0.05-0.1 µg/ml can still be detected. The measurement may be interfered by nucleic acids, nucleotides, salts or dyes, which may be present in agarose gel buffers. In the latter case method 3.1 should be used. The figure shows an UV spectrum from a 1x TAE gel buffer with 0.5 µg/ml EtBr (1) and the solution after bondEx EtBR treatment (2).

3.4 Visualisation of EtBr with the bondEX indicator cartridge

If the capacity of bondEx EtBr cartridge is exhausted EtBr breakthrough is monitored by the connected indicator cartridge. Daylight exposure shows then a red-brown ring (right picture) while under UV exposure (left picture) an intensive fluorescence is visible. Resulting from traces of EtBr weak background fluorescence may occur even before the bondEx EtBr cartridge is exhausted. This background is without importance. Check the indicator cartridge frequently and substitute it by a new one every 10-15 l flow-through and additionally if other dyes (bromophenol blue), strong background fluorescence, or contamination render the indicator cartridge useless.

4 Safety instructions – risk and safety phrases

The components of the bondEX EtBr kits do not contain hazardous contents.

Nevertheless, please watch the safety instructions given in section 2.3 regarding handling of bondEX EtBr kits.

5 Protocol for detoxification of buffer solutions

Attention: Keep cartridge vertical and remove yellow cap. Reddish brown binding matrix is covered by a foamed filter element, which may become loose during transportation. If this is the case, tap the cartridge several times onto the desk in order to compress the binding matrix and push the foamed filter disc back on the binding matrix.

1 Initially equilibration of the cartridges

- **For the first use it is necessary** to equilibrate bondEX and indicator cartridges once.

- Remove the indicator cartridge and equilibrate it separately as described below.

- Fill the bondEX EtBr cartridge once with methanol or ethanol (96-100%). Flow-through starts by gravity and should be finished within 10 min. Afterwards, fill the bondEX EtBr cartridge with water and let it run through completely.

- **Initially, it is also necessary** to equilibrate every new indicator cartridge before use once with about 1 ml of ethanol (96-100%) and then 5 ml of water. Press the solutions into the indicator cartridges using a syringe (or wash bottle) but avoid drying of cartridges.

- At last, attach the indicator cartridge to the outlet of the bondEX EtBr cartridge and close it with the appropiate cap.

2 Decontamination of EtBr solutions

- The bondEX system should consist of an equilibrated **bondEX 50 cartridge** with an attached, equilibrated **indicator cartridge which is closed by the appropiate cap**. Either a 5-10 l tank or the 1 l funnel (supplied in the starter kit) is connected to the bondEX EtBr cartridge by the appropiate adaptor (also supplied in the starter kit).

- First, **moisten the bondEX EtBr cartridge** with about 10 ml of water or gel buffer (this is not necessary if the cartridgde was already stored under water after last use. Usage of “dried” cartridges may cause decreased flowrates).

- **Apply filtered EtBr solutions** by opening the tank tap or by filling the 1 l funnel. **Remove cap** from the indicator cartridge: the **flow-through starts** and should **not exceed 20 ml/min**

(In general, if single drops can be detected flow rates are OK). Inhibiting air bubbles or minor clogging indicated by a low flow-through can be removed by tapping 2-3 x against the cartridge.

- When the solution has passed the cartridge, close the outlet of the indicator cartridge.

3 Usage and check of capacity of indicator cartridges

- The end of the EtBr binding capacity of the bondEX EtBr 50 cartridge is indicated by a colour change of the white zone in the indicator cartridges. Don´t forget to equilibrate those indicator cartridges initially and attach it to the outlet of bondEX EtBr cartridges.

- The **white zone in indicator cartridges turns from white to red when the bondEX EtBr cartridge is exhausted**: First, a red ring can be detected and the bondEX EtBr cartridge has now to be replaced. Under UV this ring can be verified by its strong, orange fluorescence.

- Other dyes or coloured contaminations like bromophenol blue may also stain indicator cartridges, thus, making it more difficult to recognize EtBr breakthrough. In consequence, (blue) **coloured or blocked indicator cartridges should be replaced immediately**!

In summary, a colour change of the indicator cartridge can have several reasons – please read the Troubeshooting in section 6.1 for additional information.

Red ring/ fluorescent ring:

- bondEX EtBr cartridge is exhausted and has to be substituted.

- The flow rate is too high and has to be regulated < 20 ml/min.

- The concentration of EtBr is too high (> 1 mg/l) and has to be diluted before decontamination.

Weak background fluorescence:

- This effect is caused by tiny amounts of EtBr passing the bondEX EtBr cartridge. Change the indicator cartridge frequently every 10-15 l.

Strong visible colour change e. g. blue / no fluorescence:

- Other dyes used in electrophoresis (e. g. bromophenol blue) will cause a coloured indicator cartridge. Change the indicator cartridge frequently e. g. every 10-15 l.

4 Storage of cartridges in use

- After use, the bondEX EtBr cartridge should be closed with the supplied end cap when attached to the adaptor (storage 1-7 days), or with the 2 different caps enclosed in the kit for long term storage (1-12 weeks).

- In order to prevent drying and microbial growth in the column, cover it with about 5 ml water (containing about 0.1 % sodium-azide for long term storage).

- **ATTENTION**: Do not store the cartridge for longer periods than 12 weeks, do not expose it to direct sunlight or to temperatures above 30 °C in order to prevent drying of the gel matrix. Exhausted cartridges should be marked by hazard labels (supplied) and collected separately in a special waste container.

Though, bondEX EtBr cartridges can be stored (see hints above), continuous decontamination from a daily refilled tank system is the best method of using bondEX EtBr cartridges!

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
No or bad flow rate	*bondEX EtBr cartridges show low or even no flow rates* • Knock 2-3 x against the bondEX EtBr cartridge in order to remove air bubbles and blocking. • Change indicator cartridge. • Check waste for precipitates or bacterial growth (filter it immediately before loading onto the bondEX system). • Use bondEX EtBr cartridges preferentially when a larger volume of waste was collected e. g. 10 l in order to maintain permanent use. • Always add some ml water to used cartridges before attaching them to the funnel, load filtered waste and finally remove outlet stopper below the cartridge. • Cartridges stored for a longer period should be loaded first with about 10 ml water in order to wet the white prefiltration element covering the red binding matrix. Soak the prefiltration element with water if the system has dried out.
Fluorescence in the indicator cartridge	*Weak background fluorescence* • Though, typically more than 99% of EtBr is removed by bondEX EtBr decontamination, residual traces of EtBr in the flow-through may lead to weak background fluorescence in the indicator cartridge. This is no problem – only strong fluorescence or a red ring as shown in section 3.4 are indicating that the bondEX EtBr cartridge has to be changed. Nevertheless, we recommend changing the indicator cartridge itself frequently and control decontaminated flow-through for presence of EtBr in order to be on the safe side.

Fluorescence in the indicator cartridge (continued)	*Weak fluorescent ring* • Under certain conditions tiny amounts of EtBr may pass the bondEX EtBr cartridge although it is not exhausted. In consequence, EtBr is bound in the indicator cartridge itself indicated by a fluorescenct ring visible under UV radiation. If this is the case, flow rates or EtBr-concentrations in the solution may be too high. A flow rate of < 20 ml/min is OK. The EtBr concentration should be optimal below 0.5 mg/ml. Alternatively, pH or salt concentration of the solutions may be far away from usual TAE/TBE buffer. Organic components (ethanol, methanol etc.) also diminish binding efficiency of bondEX EtBr cartridges. Check everything, use a new indicator cartridge and restart the decontamination process. If the fluorescent ring appears again after 1-2 l flowthough the system is exhausted and should be replaced. *(Blue) coloured indicator cartridges* • Other dyes used in electrophoresis (e. g. bromophenol blue) will cause a coloured indicator cartridge. Change the indicator cartridge. The white, dye-binding zone in the bondEX EtBr cartridge itself (see picture in section 2.1) will bind most of the dyes.

If problems arise, generally check the decontaminated waste for presence of EtBr and handle all solutions and parts of the system with care according to the toxic and mutagenic potential of EtBr.

6.2 Ordering information

Product	Cat. No.	Pack of
bondEX starter kit	740701	1 set
bondEX 50 set	740703	5 sets
bondEX folded filters XL	740705	50

6.3 Product use restriction / warranty

bondEX kit components were developed, designed and sold **for research purposes only**. No claim or representation is intended for the decontamination of any specific solution containing fluorescent staining agents. It is rather the responsibility of the user to verify the use of the **bondEX** kit for a specific application range.

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