

## JETSORB Extended Trouble Shooting Guide

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| <b>Problem: Low DNA recovery</b>              |  |
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| <u>Question / Problem</u>                     | <u>Help and comments</u>   |
| Was ethanol added to buffer A2?               | Ethanol is necessary to keep the DNA bound to the resin.<br><br>After reconstitution of buffer A2 with ethanol, the bottle must be kept <u>tightly closed</u> , if it is not in use. Otherwise the ethanol tends to evaporate, leading to a loss of the bound DNA during the washing steps with low-salt buffer. |
| Was the bottle with buffer A2 tightly closed? | If evaporation reduces the ethanol content in buffer A2, recovery of DNA will be increasingly poor. The ethanol concentration for an optimal performance of the JETSORB kit <b>must be 70% (v/v)</b> .   |

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| <p>Has the exact weight of the agarose slice been determined?</p>  | <p>For an optimal solubilization the ratio between the weight of the gel slice and the volume of binding buffer A1 should be <b>1:3</b>. Ratios higher than 1,5 : 3 will lead to an incomplete dissolving, the DNA will remain trapped in residual agarose gel and therefore DNA recovery and quality will be poor.</p>   |
| <p>Was the weight of the agarose gel slice higher than 100 mg? (see also 'Handling of larger gel slices')</p>  | <p>For gel slices with a higher weight than 100 mg <u>all</u> buffer volumes must be scaled up proportionally:</p> <ul style="list-style-type: none"> <li>a) the volume of binding buffer A1 for an optimal dissolving of the agarose gel slice and for optimal washing,</li> <li>b) the volume of buffer A2 for an optimal washing of the resin,</li> <li>c) the volume of the JETSORB suspension, <b>but this should not exceed 20 µl. This amount of JETSORB is capable of binding 10-15 µg of DNA, which should be sufficient for all applications. A larger amount of resin may trap too much liquid which may lead to an insufficient removal of residual agarose and perchlorate.</b></li> </ul> |
| <p>Were the supernatants after the binding and washing steps simply <b>poured</b> out of the reaction tube or were they <b>removed with a pipet tip</b>?</p> | <p>An incomplete removal of the supernatants during the washing steps will not only lead to poor removal of the agarose and salt contaminations in the eluate, but may also cause residual ethanol being still present during the elution step. In such a case the DNA quality is likely to be compromised.</p>   |
| <p>Has the JETSORB resin after the drying step (step 5) a homogeneous <b>snow-white</b> appearance?</p>  | <p>If not (pellet still appears 'greyish'), the drying of the resin is incomplete. This means that traces of the ethanol-containing buffer A2 are still present. Residual ethanol may reduce the efficiency of the DNA elution and will compromise the DNA quality.</p>   |
| <p>Was an oil pump-generated vacuum used for drying the JETSORB resin (step 5)?</p>  | <p>More than a <b>2 min</b> oil-pump vacuum may cause overdrying of the JETSORB resin, thus causing poor DNA recovery in the elution step. Better use a vacuum generated by a water jet filter pump for approx. 5-7 min. Anyway the drying step should be stopped when the pellet has turned completely snow-white.</p> <p><b>A good alternative for the use of vacuum is a 10 min-incubation of the JETSORB pellet at 50-60 °C in a thermo bloc or water bath with the cap open after removing the buffer A2 supernatant of the final washing step.</b></p>  |

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| <p>Fragment smaller than 500 bp?</p>  | <p>JETSORB works well for dsDNA fragments as small as 50 bp. But there are several aspects which may raise problems with small fragments.</p> <p>First, the typical amount of small DNA fragments is quite low.</p> <p>Second, and most important, the diffusion rate for small DNA fragments in agarose gels is higher than for larger ones, so that losses may occur by diffusion into the overlaying buffer or by band-broadening.</p> <p>Third, for small fragments the yield may decrease due to denaturation caused by elevated temperatures and/or the presence of chaotropic salt. ssDNA is <b>not bound well</b> by JETSORB. So, if the fragment size is 500 bp or even smaller, do the solubilization of the agarose (step 1 of the protocol) at ambient temperature for an extended period of time with repeatedly vortexing the tube.</p> <p>It may be helpful to minimize the buffer overlayer on the gel and to minimize the migration distance during electrophoresis.</p> |
| <p>Fragment larger than 5 kb?</p>   | <p>Using the standard protocol, the recovery rate of the DNA may decrease slightly for fragments being larger than 5 kb in size. There are several modifications mentioned in the protocol to avoid a poor recovery for larger fragments:</p> <ol style="list-style-type: none"> <li>1. Extend the incubation time during elution (to up to 15 min).</li> <li>2. Raise the elution temperature to 60°C.</li> <li>3. Don't vortex. Shearing of large fragments can be avoided if the JETSORB matrix is resuspended by flicking the tube with the finger tip or by pipetting up and down with a wide-bored pipet tip.</li> <li>4. Don't overdry the JETSORB pellet after the final washing step with buffer A2. To ensure this, don't use vacuum. <b>A good alternative for the use of vacuum is a 10 min-incubation of the JETSORB pellet at 50-60°C in a thermo bloc or water bath with the cap open after removing the buffer A2 supernatant of the final washing step.</b></li> </ol>   |
| <p>Was the DNA to be eluted supercoiled?</p>  | <p>Supercoiled DNA (e.g. undigested plasmids) can principally be bound to and eluted from the JETSORB resin but <b>cannot be extracted from agarose gels with the JETSORB kit.</b></p>  |
| <p>Does your JETSORB pellet still smell of ethanol after the drying step following the final wash with buffer A2?</p> | <p>Ethanol, which is present in buffer A2, may reduce the overall elution efficiency. In particular, elution of large fragments is affected. After drying, the JETSORB pellet must not longer appear 'greyish' in any way, but <b>completely snow-white.</b></p>  |

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| Do you work with AT-rich DNA?                       | AT-rich DNA (>70% AT content, i.e. intron-DNA) denaturates easier (which means separation into its two DNA strands) than "normal" DNA. Denaturation is enhanced by elevated temperatures and the presence of chaotropic salt. So do the solubilization step of the agarose (step 1 of the protocol) at <u>ambient</u> temperature for an extended period of time (approx. 20-30 min) with repeatedly vortexing the tube. |
| What is the storage temperature of the JETSORB kit? | Several competitor's products are recommended to be stored cold at 4°C if they are not in use.<br><br>JETSORB kits should be kept at <b>ambient temperature</b> . All buffers and ingredients of JETSORB are completely different from other product's components. There is absolutely no need for a storage in the cold.  |
| Suggestion (no user question)                       | Elution with 40 µl of TE buffer or water per 10 µl of JETSORB suspension may increase the recovery by 15%.   |

| <b>Problem: Handling of larger gel slices</b>                          |   |
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| <u>Question / Problem</u>  | <u>Help and comments</u>  |
| Was the weight of the agarose gel higher than 100 mg?                  | For gel slices with a higher weight than 100 mg <u>all</u> buffer volumes must be scaled up proportionally:<br>a) the volume of binding buffer A1 for an optimal dissolving of the agarose gel slice and for optimal washing,<br>b) the volume of buffer A2 for an optimal washing of the resin,<br>c) the volume of the JETSORB suspension, <b>but this should not exceed 20 µl. This amount of JETSORB is capable of binding 10-15 µg of DNA, which should be sufficient for all applications. A larger amount of resin may trap too much liquid which may lead to an insufficient removal of residual agarose and perchlorate.</b> |
| Did you melt your agarose gel slice in a glass tube?                   | <b><u>This must not be done!!</u></b> Due to the presence of the chaotropic salt in the solubilization process the DNA binds to the wall of the glass tube and will be lost.  |
| Was the larger agarose gel slice <b><u>completely</u></b> solubilized? | Larger gel slices take longer to dissolve in buffer A1. <b>Therefore extend the solubilization step for 10-15 min.</b> The solubilization can be enhanced by cutting down the large gel slice to smaller pieces. If the size of the DNA fragment that is to be purified is not too small (<500 bp), the temperature for solubilization can be increased to <b>55°C</b>  |

| <b>Problem: Agarose gel slice doesn't solubilize</b> |  |
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| <u>Question / Problem</u>                            | <u>Help and comments</u>   |
| What was the buffer A1-to-gel-ratio?                 | The standard protocol is designed for gel concentrations of up to <b>2% (TBE buffer)</b> or <b>2.5% (TAE buffer)</b> . For higher gel concentrations use a higher buffer-to-gel ratio (e.g. 500 µl of buffer A1 for 100 mg of agarose gel slice).<br>If the buffer A1-to-gel ratio is too low, the gel doesn't solubilize completely leading to <b>poor DNA recovery and quality</b> . Determine the weight of the gel slice exactly with a balance and add the correct volume of buffer A1. |
| What was the temperature used for solubilization?    | If the solubilization step is carried out at temperatures below 50°C, <b>the time required for completely dissolving the agarose will increase</b> . The gel should only be solubilized at room temperature if low agarose concentrations ( $\leq 1\%$ ) are used, or if one is working with small and/or AT-rich DNA fragments. DNA binding to the JETSORB matrix is not affected at room temperature.  |
| Was your piece of agarose larger than 100 mg?        | If your agarose gel slice is larger than 100 mg it should be cut down to small pieces in order to enhance solubilization.  |

| <b>Problem: Formation of a precipitate after addition of buffer A1</b> |  |
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| <u>Question / Problem</u>  | <u>Help and comments</u>   |
| Did your assay contain SDS?  | SDS, which may be present from proceeding steps in concentrations of more than 0.1% will precipitate in solutions containing high concentrations of sodium perchlorate. A precipitate of small amounts of SDS (up to 1% SDS in the original assay) will be removed during the washing steps with buffer A2 and can be tolerated. |
| Did your assay contain potassium ions?                                 | Potassium perchlorate is highly insoluble. If your assay contains potassium ions, add first buffer A1 (but not yet the JETSORB resin) and spin down the resulting pellet of potassium perchlorate. Transfer the supernatant to a new tube and then add the JETSORB resin.  |

| <b>Problem: Slow drying of the JETSORB resin after the final wash with buffer A2</b>      |  |
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| <u>Question / Problem</u>   | <u>Help and comments</u>   |
| Did you simply <b>pour out</b> the supernatant of buffer A2 after the final washing step? | <b>Remove the last traces of buffer A2 with a small pipet tip.</b> Take care of residual buffer backdraining from the wall of the tube. Accelerate drying by vacuum, but don' t overdry the pellet. The pellet will be dry if it appears snow-white. Even parts of the pellet <b><u>must not</u></b> appear ' greyish' any longer.   |
| Was vacuum used to enhance drying?  | Vacuum should be used very carefully. Using a speed vac with an oil pump should not exceed 1-2 min, using a water jet filter pump approximately 5-7 min.<br><b>A good alternative for the use of vacuum is a 10 min-incubation of the JETSORB pellet at 50-60°C in a thermo bloc or water bath with the cap open after removing the buffer A2 supernatant of the final washing step.</b> |

| <b>Problem: Insoluble pellet after ethanol precipitation</b>   |  |
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| <u>Question / Problem</u>  | <u>Help and comments</u>   |
| Were the supernatants after the binding and washing steps simply <b>poured out</b> of the reaction tube or were they removed with a pipet tip? | <b>The supernatants after the binding and washing steps must be removed <u>quantitatively</u>.</b> Incomplete removal will lead to a poor removal of agarose and to significant remainders of perchlorate. These contaminants can only be removed if the procedure is repeated (take 100 µl of ' dirty eluate' instead of 100 mg of agarose gel slice).  |
| Has the assay been scaled up?  | 1. Higher amounts of JETSORB resin take longer to resuspend. If the resin is not completely resuspended contaminants may not be efficiently removed.<br><br>2. Depending on the quality, trace amounts of certain contaminants derived from the agarose may bind to the JETSORB resin. To minimize the potential binding of the contaminants to the resin add the JETSORB-suspension only after complete solubilization of the agarose so that the resin is loaded at a lower degree with agarose. |
| Type or vendor of agarose changed?   | Agaroses of lower quality are more likely to lead to problems with contaminations.   |

| <b>Problem: Thick white interphase after additional phenol/chloroform extraction</b>   |   |
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| <u>Question / Problem</u>  | <u>Help and comments</u>  |
| Were the supernatants after the binding and washing steps simply <b>poured out</b> of the reaction tube or were they removed with a pipet tip? | <p><b>The supernatants after the binding and washing steps must be removed <i>quantitatively</i>.</b> Incomplete removal will lead to a poor removal of agarose and to significant remainders of perchlorate.</p> <p>These contaminants can only be removed if the procedure is repeated (take 100 µl of 'dirty eluate' instead of 100 mg of agarose gel slice).</p>  |
| Has the assay been scaled up?  | <p>1. Higher amounts of JETSORB resin take longer to resuspend. If the resin is not completely resuspended contaminants may not be efficiently removed.</p> <p>2. Depending on the quality, trace amounts of certain contaminants derived from the agarose may bind to the JETSORB resin. To minimize the potential binding of the contaminants to the resin add the JETSORB-suspension only after complete solubilization of the agarose so that the resin is loaded at a lower degree with agarose.</p> |
| Type or vendor of agarose changed?   | Agaroses of lower quality are more likely to lead to problems with contaminations.  |

| <b>Problem: Dried-out JETSORB resin</b> |   |
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| <u>Question / Problem</u>               | <u>Help and comments</u>  |
| Has the suspension already been used?   | <p>We observed that the suspension will not dry out during storage (even after one year). But if the tube has been opened several times to take out aliquots of the resin, beads may be trapped between the rim of the tube and the tube's cap. Additionally, if the tube is not tightly closed, evaporation of the liquid can occur.</p> <p>As a consequence there must be to paid attention <b>that there is no resin at the tube's rim that can be trapped between the rim and the cap</b> or to <b>remove such resin before closing the tube</b>. Nevertheless, a dried-out resin can still be used:</p> <p>The resin is supplied as an approx. 65 % (v/v) slurry. In order to reconstitute the dried-out resin, give the vial a quick spin, estimate the height of the resin in the vial, and add half the volume of deionized water. The JETSORB resin is not damaged at all by dehydration.</p> <p><i>General guidelines are:</i></p> <ul style="list-style-type: none"> <li>-Store the vial with the resin upright. Take out aliquots of the JETSORB resin without contaminating the rim of the vial,</li> <li>-seal the cap of the vial with parafilm, or</li> <li>-carefully remove remnants of the JETSORB resin from the rim of the vial with a clean Kimwipe.</li> </ul> |

| <b>Problem: JETSORB beads in the eluate</b>                  |   |
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| <u>Question / Problem</u>                                    | <u>Help and comments</u>  |
| Are JETSORB particles carried over together with the eluate? | <p>We have extensively tested JETSORB contaminations in enzymatic reactions. We have found that only amounts of JETSORB beads causing a visibly turbid eluate may partially inhibit enzymatic reactions.</p> <p>To perfectly avoid getting JETSORB beads into the eluate follow these simple steps:</p> <ol style="list-style-type: none"> <li>a. centrifuge for 2 minutes at top speed;</li> <li>b. remove supernatant <u>immediately</u> after the centrifuge has stopped;</li> <li>c. avoid processing too many samples in parallel.</li> </ol> <p>Alternatively, the eluate can be filtered through a micro-spin device with a 0.45 µm membrane (e.g. Millipore Ultrafree-MC Filter Unit, Durapore membrane, 0.45 µm) to get rid of any residual beads.</p> |

| <b>Problem: Subsequent enzymatic reactions with the JETSORB-purified DNA are inhibited</b> |
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| <u>Question / Problem</u>   | <u>Hints and suggestions</u>   |
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| <p>Did you <b>resuspend</b> the pelleted JETSORB resin between each washing step?</p>   | <p><b>May sound basic, but this is obviously vital!!!</b> The washing efficiency depends on:</p> <ol style="list-style-type: none"> <li>1. complete resuspension of the beads so that adhering contaminations can be washed away.</li> <li>2. complete removal of the supernatant so that the diluting-out effect for the initially present agarose and the high-salt buffer A1 is maximal.</li> </ol>   |
| <p>Were the supernatants after the binding and washing steps simply <b>poured out</b> of the reaction tube or were they removed with a pipet tip?</p> | <p><b><u>This isn't recommend for the JETSORB kit!!!</u></b> The supernatants <b>after the binding and washing steps must be removed <u>quantitatively</u> with a pipet tip immediately after centrifugation. <u>Take care of residual liquid backdraining from the wall of the tube.</u></b></p> <p>Incomplete removal of the supernatants will lead to poor removal of agarose and perchlorate, thus compromising the quality of the purified DNA.</p> <p>These contaminants can only be removed if the JETSORB procedure is repeated on the eluate (take 100 µl of 'dirty eluate' instead of 100 mg of agarose gel slice) and proceed as described in the protocol.</p>   |
| <p><b>General Suggestions</b></p>   | <p><b><i>see below</i></b></p>   |
| <p><i>Contamination with JETSORB particles</i></p>  | <p>As JETSORB particles may interfere with enzymatic reactions, downstream applications with JETSORB-purified DNA may be affected.</p> <p>To avoid this, remove the supernatant after the elution very carefully:</p> <ul style="list-style-type: none"> <li>- Centrifuge at least 30 sec at full speed in a table top centrifuge.</li> <li>- Remove supernatant immediately.</li> <li>- Don't process too many samples at a time. The JETSORB pellet, although being quite stable directly after the centrifugation, may become unstable during extended standing on the bench.</li> <li>- Allow 2 - 4 µl of eluate to remain on the pellet.</li> <li>- Alternatively spin the eluate with the JETSORB beads through a micro-spin device with a 0.45 µm membrane (see above). This will remove all residual beads and give you nearly 100% recovery.</li> </ul> |
| <p><i>Reactions, which are sensitive to NaClO<sub>4</sub></i></p>   | <p>If the washing steps with buffer A2 are not carried out properly, the final concentration of NaClO<sub>4</sub> in the eluate may reach concentrations higher than 50 mM. This may compromise the activity of certain enzymes. To avoid this:</p> <ol style="list-style-type: none"> <li>1. remove the supernatants of the washing steps <b><u>quantitatively with a pipet tip.</u></b></li> <li>2. Optionally, wash 3 times with buffer A2.</li> </ol>  |

*Reactions, which are sensitive to ethanol*

Dry the JETSORB pellet until all traces of ethanol have evaporated. This can be enhanced by removing the supernatant quantitatively with a small pipet tip after the final washing step.

With DNAs of less than 5 kb in size the JETSORB pellet can be dried under vacuum. Avoid overdrying (see suggestions above)!

**A good alternative for the use of vacuum is a 10 min-incubation of the JETSORB pellet at 50-60 °C in a thermo bloc or water bath with the cap open after removing the buffer A2 supernatant of the final washing step.**

*Reactions, which are sensitive to NaCl and EDTA*

If the supernatant is not completely removed after the final washing step the salt concentrations in the eluate for sodium chloride and/or EDTA may reach concentrations, that may compromise the activity of certain enzymes.

To avoid this:

1. remove the supernatants during all washing steps quantitatively with a pipet tip.
2. Use 10 mM Tris/HCl, pH 8.0 without EDTA for elution

## Some inquiries that came from customers

### Smaller elution volumes?

Elution of DNA can be carried out with only 10 µl of elution buffer per 10 µl JETSORB suspension. In this case, reduced recovery-rates of about 60 % have to be accepted.

As an alternative spin the JETSORB bead/buffer mixture through a 0,45 µm spin filter. This will give you nearly 100% recovery.

### Elution in water?

Elution with sterile water is possible, but in some cases recovery-rates of larger fragments may be lower. DNA dissolved in water is less stable than in TE or 10 mM Tris (pH 8.0) buffer.

### What are the minimum, what the maximum sizes of the DNA fragments I can elute from my gel?

Lower limit: 25-30 bp, upper limit: 45 kbp. Below 50 bp and above 15 kbp reduced recoveries must be accepted. Be careful when handling DNA' s with size  $\geq$  15 kb. **Don't vortex** to resuspend the matrix, because shearing forces may affect the DNA. Resuspend the JETSORB resin by pipetting up and down slowly with a wide-bored pipet tip.

For single stranded DNA and RNA the lower size limit is about 200 nt.

### How much DNA can I isolate ?

10 µl JETSORB resin have a nominal capacity of approximately 7.5 µg of DNA.

### Ligation problems

The problem of poor efficiencies in fragment ligation is caused in most cases by an incomplete removal of the supernatants during the washing steps. Solubilized agarose and perchlorate will not be totally removed and thus may interfere with ligation or other enzymatic reactions. **So, always remove supernatants quantitatively (pipet tip!) for best ligation results!**

### My DNA is very AT-rich; does the sequence influence the recovery with JETSORB?

If a double-stranded DNA is AT-rich (average AT-content  $\geq$  70%), it is possible that the dsDNA melts into its single strands. This may happen during solubilization of the gel or during elution of the fragments, because in perchlorate-containing solutions as well as in buffers without any salt the melting temperature of the DNA-double strand is reduced. As a consequence, the DNA is poorly visible on agarose gels and cloning and enzymatic reactions will become impossible.

To avoid this, the temperature should be reduced to 37°C or even lower during solubilization of the gel and during elution of the fragments. To compensate for the lower temperatures the incubation times must be extended. This should only be done if the gel concentration is  $\leq$  1% (see above).

### **Can I isolate DNA/RNA-heteroduplexes from agarose gels?**

Two of our customers in Germany have successfully used JETSORB for the isolation of cDNA first strand synthesis products (RNA/DNA hybrids). Their information is that the recovery is good and that the quality is well-suited for further enzymatic manipulations (e.g. second strand-synthesis of cDNA).

### **Can I isolate RNA/RNA-homoduplexes from agarose gels?**

Isolation of RNA/RNA homoduplexes has not been tested in detail, yet. The isolation of ribosomal RNA, which is partially double-stranded, works fine.

### **Lower grade agaroses**

We have tested JETSORB with the GIBCO-BRL and the FMC product-line. It is not necessary to use special DNA-grade agaroses (e.g. SeaKem GTG Agarose) or low gelling-temperature agaroses (e.g. NuSieve). The lower quality FMC SeaKem LE, HE and LE or BRL ultrapure agaroses perform equally well. For other brands or qualities no statement can be given.

Much more critical is the incomplete removal of the supernatants during the washing steps: solubilized agarose and perchlorate from buffer A1 will not be totally washed away and may interfere with subsequent enzymatic reactions if not removed properly. **So, always remove supernatants quantitatively with a pipet tip for best results!**

### **Polyacrylamide gels**

JETSORB can not directly be used in combination with polyacrylamide gels. This is because the chaotropic salt in buffer A1 is able to dissolve the non-covalent linkages between the agarose molecules, but as polyacrylamide is a **covalently linked** network, this method for solubilizing the gel matrix can't be used.

### **What your client can TRY is the following:**

- a. Grind the gel to fine pieces (e.g. squash the gel slice between a blue pipet tip and the wall of the reaction tube).
- b. Add a twofold volume of TE buffer and incubate for 1 hr at 50 °C. Shake several times during incubation. The DNA fragments will leave the polyacrylamide matrix by diffusion and go over into the surrounding TE buffer. DNA fragments of smaller size will leave the gel more efficiently than larger ones.
- c. Centrifuge briefly and transfer the eluate to a new reaction tube. To keep DNA losses as small as possible, collect the supernatant as quantitative as possible. A very easy method to do this, is transferring the mixture from step b into a micro-spin tube with a 0.45 µm membrane (i.e. polysulfone). The liquid will then be separated from the solid polyacrylamide matrix by a 5-min centrifugation step at full speed in a conventional microfuge.

- d. For each 100  $\mu$ l of eluate add 300  $\mu$ l of buffer A1 and 10  $\mu$ l of JETSORB suspension.  
**Anyway, the maximum amount of JETSORB suspension should not exceed 20  $\mu$ l (see comments above).**
- e. Allow the DNA to bind to the matrix for 5 min. at room temperature.
- f. Proceed as described in the JETSORB protocol beginning with step 3 (' high-salt wash' ). The volumes of the buffers in all washing steps must be scaled up according to step d.

As the elution of DNA fragments from polyacrylamide gels was not the major goal during the development of the JETSORB kit, this procedure has not been extensively tested with subsequent biochemical reactions. Therefore we can not guarantee any success.