

Product List

Product	Prep.	Contents	Cat. #
JETSORB Gel Extraction Kit / 150	150	1.5 ml JETSORB Solution A1 Solution A2	110150
JETSORB Gel Extraction Kit / 300	300	3.0 ml JETSORB Solution A1 Solution A2	110300
JETSORB Gel Extraction Kit / 600	600	6.0 ml JETSORB Solution A1 Solution A2	110600

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Protocol

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JETsorb

DNA Extraction from Agarose Gels

pure, fast convenient,
competitive.....

Connected with:

PCR

Ligation

Sequencing

Transcription

Labeling etc.



Protocol

Before use, reconstitute buffer A2

1. Solubilization of agarose (HM or LM agarose)

Excise the agarose gel slice, determine its weight and transfer the slice into a suitable tube. **For each 100 mg gel slice** add the following kit components:

- 300 µl buffer A1
- 10µl JETSORB suspension

Comments: For larger gel slices (>100 mg) all kit buffers (buffer A1, buffer A2 and the JETSORB suspension) must be **scaled up proportionally**. The ratio of gel slice weight to volumes of buffers A1 and A2 is always 1 to 3 (w/v). **Before use, resuspend JETSORB carefully by vortexing.**

2. DNA Binding

Vortex and incubate the assay at **50°C for 15 min**. Mix every 3 min during incubation. After this, centrifuge (**30 sec / ≥10,000 g**) and remove the supernatant completely with a pipette.

Comments: Large gel slices (more than 300 mg) take slightly longer to dissolve. When the gel has completely melted, continue incubation for an additional 5 min. To avoid sedimentation of JETSORB, mix the assay every 3 min during incubation.

3. High Salt Wash

Wash (resuspend) the pellet once with **300 µl of buffer A1**. Centrifuge as before to recover JETSORB and remove the supernatant with a pipette. Resuspend the pellet by vortexing and/or flicking the tube.

Comments: The JETSORB pellet is very compact, so that the supernatant can be removed easily with a pipette. It is vital, regarding the quality

of the DNA that any wash buffer is removed quantitatively. Make sure that the wash buffer that runs back from the wall of the tube is also removed. **Vortexing is only recommended for DNA fragments up to 5 kb.**

4. Low Salt Wash

Wash (resuspend) the pellet with **300 µl of reconstituted buffer A2**. Centrifuge as before and remove the supernatant with a pipette. **Repeat step 4!**

Comments: For reconstitution of buffer A2, see label on the bottle. As mentioned in the comments on step 3, it is vital, regarding the quality of the purified DNA that any wash buffer is removed quantitatively. Make sure that wash buffer that runs back from the wall of the tube is also removed and that the JETSORB resin has no liquid supernatant. **After use, close bottle A2 tightly.**

5. JETSORB Drying

Dry the JETSORB pellet **by air or under vacuum**.

Comments: Before DNA elution, the pellet must be free of ethanol. The pellet turns **snow white** when it is completely dry (using a speedvac approx. 1-2 min, using a vacuum chamber with a Water Jet Filter Pump approx. 5-7 min). Low yields can result when JETSORB has been overdried. **Be careful when using a speed vac.** A very good alternative is an incubation of the reaction tube for 10 min at 50°C with the cap open in a thermo bloc or a water bath.

6. DNA Elution

Add 20 µl TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) or water, resuspend the pellet as before and incubate for **5 min at 50°C**. Flick the tube **once** during incubation. Centrifuge as before and transfer the supernatant into a new tube.

Comments: After centrifugation, save the supernatant very carefully to avoid JETSORB beads getting into the transferred supernatant. Contaminating beads may inhibit enzymatic reactions. **The DNA is ready to use for all kind of reactions.**

Trouble-Shooting Guide

When yields are low!

The protocol is designed to achieve very high yields of more than 80%, depending on the size and amount of the DNA to be extracted. If low yields occur, they might be associated with:

1. **Incorrect ratio of gel slice to JETSORB suspension** (see protocol & comments step 1).
2. **Incomplete solubilization of the gel slice** during step 2 (see protocol & comments step 2).
3. **Incorrect temperatures during DNA elution:**
If the temperature is too low during DNA elution, yields decrease dramatically, especially for long DNA fragments.
4. **Yields of large DNA fragments** may increase, when the incubation is prolonged (up to 15 min) or elution temperature is increased to 60°C.
5. **Supercoiled or circular DNA (plasmid DNA):**
The kit is not designed to extract supercoiled plasmid DNA from agarose gels.

When enzymatic reactions are inhibited!

After elution the DNA is ready to use for all reactions. Unsatisfactory results can be caused by the following:

1. **Residual ethanol in the sample** after DNA elution (see protocol & comments step 4).
2. **JETSORB beads contaminate** the transferred DNA solution and inhibit enzymatic reactions. To avoid this, centrifuge the eluate again and transfer the supernatant into a clean tube.
3. **Residual salt in the sample** after DNA elution is due to the incomplete removal of supernatants after washing steps.

Solutions

Reconstitution of solution A2

The bottle of solution A2 contains concentrated buffer. Before use, add ethanol (95-100 % ethanol) as mentioned on the bottle's label.

<u>JETSORB Suspension</u>	Store at RT
<u>Solution A1*</u> contains concentrated NaClO ₄ , TBE-Solubilizer, sodium acetate	Store at RT
<u>Solution A2 (reconstituted)</u> contains ethanol, NaCl, EDTA and Tris/HCl	Store at RT
	RT = room temperature
* Solution A1 contains sodium perchlorate, use with proper precaution.	

Additional Data and Comments

- The DNA capacity of JETSORB is 7.5 µg / 10 µl JETSORB suspension.
- Yields can be increased by about 10% if a second elution with 40 µl TE buffer follows.
- The DNA elution can be performed with 10 µl TE buffer if recoveries of 60% can be tolerated.



Notes



Notes

