

**User-developed  
protocol**

## User-Developed Protocol:

### Regeneration of Oligotex<sup>®</sup> resin from the Oligotex mRNA Kit

This procedure has been adapted by customers for regeneration of QIAGEN<sup>®</sup> Oligotex<sup>®</sup> resin, and is for use with the Oligotex mRNA Kits. **It has not been thoroughly tested and optimized by QIAGEN.**

The procedure has been used successfully by customers for regeneration of Oligotex resin from spin columns following elution of poly A<sup>+</sup> mRNA.

Please be sure to read the *Oligotex Handbook* and the detailed Spin-Column Protocol carefully before beginning this procedure.

### Procedure

1. Transfer spin column containing the Oligotex resin upside-down into a 15 ml tube.
2. Add about 3 ml 0.1 M NaOH; 1 mM EDTA, so that the column is completely covered with liquid.
3. Leave at room temperature for 10 min, vortex and centrifuge for 5 min at maximum speed. Discard the spin column, and aspirate supernatant.
4. Resuspend Oligotex resin in 1 ml 0.1 M NaOH, transfer into a 1.5 ml microcentrifuge tube, centrifuge for 5 min at maximum speed in a microcentrifuge (14,000–18,000 x g for most microcentrifuges), and aspirate supernatant.
5. Resuspend Oligotex resin in 500 µl of 10 mM Tris-Cl pH 7.0, 1 mM EDTA (TE) that also contains 0.1% SDS (or 0.1% Nonidet<sup>®</sup> P-40, Tween<sup>®</sup> 20, or Triton<sup>®</sup> X-100). Centrifuge as above and aspirate the supernatant.
6. Repeat step 5 until the pH of the supernatant is neutral.
7. Resuspend Oligotex resin in 10 mM Tris-HCl pH 7.0, 1 mM EDTA (TE) that also contains 0.1% SDS (or 0.1% Nonidet P-40, Tween 20, or Triton X-100). Alternatively, resuspend in 1 x Binding Buffer (500 mM NaCl; 10 mM Tris-HCl, pH 7.0; 1 mM EDTA). Resuspend in the original volume.

**Note:** The suspension must be pH-neutral. If it is alkali, mRNA will degrade.

During the aspiration steps of the poly A<sup>+</sup> mRNA purification procedure or the regeneration procedure, some of the Oligotex resin may be lost. When reusing Oligotex suspension, the volume of Oligotex resin should therefore be increased in proportion to the loss (approximately 10%). Alternatively, resuspend Oligotex resin with 90% of the original volume.

Regeneration of Oligotex resin is dependent on the conditions of use. Therefore, the quality of regenerated Oligotex resin is not guaranteed.

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## Regeneration of Oligotex resin (batch procedure)

This procedure has been adapted by customers for regeneration of QIAGEN Oligotex resin and is for use with the Oligotex Direct mRNA Kits, the Oligotex mRNA Kits, or Oligotex Suspension. **It has not been thoroughly tested and optimized by QIAGEN.**

The procedure has been used successfully by customers for regeneration of Oligotex resin following elution of poly A<sup>+</sup> mRNA.

Please be sure to read the *Oligotex Handbook* and the detailed Spin-Column Protocol carefully before beginning this procedure.

## Procedure

1. Resuspend Oligotex resin in 1.2 ml 0.1 M NaOH; 1 mM EDTA.
2. Leave at room temperature for 10 min, centrifuge for 5 min at maximum speed in a microcentrifuge (14,000–18,000 x g for most microfuges), and aspirate supernatant.
3. Repeat steps 1 and 2.
4. Resuspend Oligotex resin in 500 µl 10 mM Tris-Cl, pH 7.0; 1 mM EDTA (TE) that also contains 0.1% SDS (or 0.1% Nonidet P-40, Tween 20, or Triton X-100). Centrifuge as above and aspirate the supernatant.
5. Repeat step 4 until the pH of the supernatant is neutral.
6. Resuspend Oligotex resin in 10 mM Tris-Cl, pH 7.0; 1 mM EDTA (TE) that also contains 0.1% SDS (or 0.1% Nonidet P-40, Tween 20, or Triton X-100) Alternatively, resuspend in 1 x Binding Buffer (500 mM NaCl; 10 mM Tris-Cl, pH 7.0; 1 mM EDTA). Resuspend in the original volume.

**Note:** The suspension must be pH-neutral. If it is alkali, mRNA will degrade.

During the aspiration steps of the poly A<sup>+</sup> mRNA purification procedure or the regeneration procedure, some of the Oligotex resin may be lost. When reusing Oligotex suspension, the volume of Oligotex resin should therefore be increased in proportion to the loss (approximately 10%). Alternatively, resuspend Oligotex resin with 90% of the original volume.

Regeneration of Oligotex resin is dependent on the conditions of use. Therefore, the quality of regenerated Oligotex resin is not guaranteed.

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Trademarks: QIAGEN<sup>®</sup>, Oligotex<sup>®</sup> (QIAGEN); Nonidet<sup>®</sup> (Shell Chemicals); Triton<sup>®</sup> (Rohn & Haas Inc.); Tween<sup>®</sup> (ICI Americas, Inc.).

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