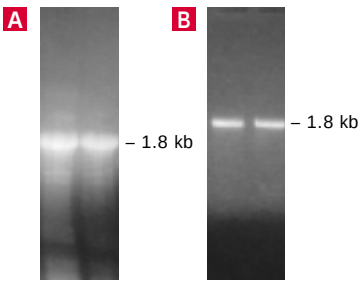
QIA-Hints

QIAGEN Technical Services are always available to answer your questions!

RNA

Q Can I use a QIAGEN® kit to extract RNA from a formaldehyde agarose gel?



A Yes. The QIAquick® Gel Extraction Kit for extraction of DNA from gels can also be used for RNA gel extraction. The protocol was kindly provided by J. Knobloch, Heinrich Heine University, Düsseldorf, Germany.*

1. Excise the RNA fragment from the formaldehyde agarose gel with a clean, sharp scalpel.
2. Weigh the gel slice, and record the weight. Soak the gel slice in TE buffer for 25 min at room temperature with gentle shaking.
3. Remove the gel slice from the TE buffer, and place it in a colorless tube. Add 6 volumes of Buffer QG to 1 volume of gel, based on the gel weight (100 mg ~ 100 µl).
4. Incubate at 58°C for 25 min. To help dissolve the gel, mix by vortexing the tube every 2–3 min during the incubation.
5. Continue the QIAquick Gel Extraction Kit Protocol (using a microcentrifuge) in the *QIAquick Spin Handbook*, beginning with step 4.

**Please note that this is a user-developed protocol. QIAquick Gel Extraction Kits are not guaranteed to be RNase-free.*

Figure 1 ***A*** *Total RNA was isolated from the parasitic blood fluke* Schistosoma mansoni *using the RNeasy® Mini Kit and run on a formaldehyde agarose (1.2%) gel. (Note: The 28S rRNA in* S. mansoni *contains a break site so that the rRNA splits into two parts, which run on a gel at the same size as the 18S rRNA.)* ***B*** *The rRNA bands were excised and treated as described above (left lane) or using 10 volumes of Buffer QG in step 3 (right lane). The extracted RNA was then analyzed on a new formaldehyde agarose gel. (Data kindly provided by J. Knobloch, Department of Genetic Parasitology, Heinrich Heine University, Düsseldorf, Germany.)*

PROTEIN

Q I want to purify a 6xHis-tagged protein, but I have two problems:

1. The expression rate is low, and so I have only a few micrograms of my protein.
2. Purification is difficult because of the low concentration of my protein.

How can I efficiently purify my protein?

A When working with small amounts of 6xHis-tagged protein in dilute solution, such as proteins expressed in mammalian cells or secreted into cell-culture medium, we recommend using Ni-NTA Magnetic Agarose Beads.

The binding capacity of 1 ml Ni-NTA Magnetic Agarose Bead suspension is 300 µg of 6xHis-tagged protein. Adjusting the amount of Ni-NTA Magnetic Agarose Beads to accommodate the total amount of 6xHis-tagged protein present in the solution (e.g., 10 µl Ni-NTA Magnetic Agarose Beads for up to 3 µg of 6xHis-tagged protein) improves the purity of the eluted protein since using too much matrix for purification may lead to copurification of nontagged proteins. This is especially true when purifying proteins from lysates containing complex mixtures of proteins with multiple histidine residues or metal-binding motifs.

Even very small amounts of the strongly magnetic beads can be collected magnetically with extremely high efficiency from large volumes, leading to a high recovery rate of purified protein.

The small elution volumes (as little as 25 µl) result in highly concentrated protein solutions. This allows detection of the purified proteins even on Coomassie® stained SDS polyacrylamide gels.

Ni-NTA Magnetic Agarose Beads combine the benefits of Ni-NTA and magnetic-bead technology making them highly suited for the purification of 6xHis-tagged protein from dilute solutions (up to 50 ml). For more detailed information, contact QIAGEN Technical Services.

Please do not hesitate to call your local QIAGEN Technical Service Department if you have any questions or require further information regarding any of the QIAGEN products mentioned above.