

Overview of Silica Adsorption

This chapter describes the High Pure kits and other kits which use silica adsorption to quickly and simply purify small amounts of nucleic acid. All of them use methods that:

- Can process multiple samples in minutes, rather than hours or days
- Require less handling of potentially hazardous samples
- Eliminate phenol extraction, precipitation, and other nucleic acid handling steps that can lead to loss or fragmentation of the desired product

For a quick overview of these products, continue reading this article. Or, for detailed information on the product most relevant to your research, turn to the page that describes the product in detail:

If you are interested in	For preparing	See page
High Pure PCR Template Preparation Kit	Genomic DNA from small amounts of whole blood; buffy coat; cultured cells; tissue; mouse tail; gram positive or gram negative bacteria; or paraffin-embedded, fixed tissue sections	21
High Pure PCR Cleanup Micro Kit	Reaction products by eliminating contaminants from the reaction mixes or DNA products from agarose gel slices	33
High Pure PCR Product Purification Kit	Product DNA (a few hundred bp to 50 kb) from 100 µl PCR or other enzymatic reactions, or 100 mg agarose gel slice	40
High Pure Plasmid Isolation Kit	Purified plasmid DNA from 0.5 – 4.0 ml cultures of <i>E. coli</i>	47
High Pure RNA Isolation Kit	Intact total RNA from small amounts of whole blood, cultured cells, yeast, gram positive or gram negative bacteria	53
High Pure RNA Tissue Kit	Intact total RNA from tissues	62
High Pure FFPE RNA Micro Kit	Total RNA from 1 – 10 µm FFPE tissue sections	67
High Pure RNA Paraffin Kit	Total RNA from fresh-frozen and formalin-fixed, paraffin-embedded tissue sections up to 20 µm	73
High Pure Viral RNA Kit	Intact viral RNA from 200 – 600 µl of serum, plasma, cell culture supernatant, tears, urine, or breast milk	82
High Pure Viral Nucleic Acid Kit	Total viral nucleic acids (DNA and RNA) from 200 – 600 µl of serum, plasma, whole blood, or cell culture supernatant	87
High Pure 16 System Viral Nucleic Acid Kit	Total viral nucleic acids (DNA and RNA) from 200 – 600 µl of serum, plasma, or cell culture supernatant	92
Agarose Gel DNA Extraction Kit	Product DNA (0.4 – 100 kb) from 100 – 200 mg agarose gel slice	108
High Pure 96 UF Cleanup Kit	Product DNA by high throughput ultra filtration	113

Principle of silica adsorption

All the kits described in this chapter depend on the tendency of nucleic acids to adsorb to silica (glass) in the presence of a chaotropic salt such as sodium iodide (NaI), guanidine thiocyanate or guanidine hydrochloride (Melzak et al., 1996).

This tendency was discovered by Vogelstein and Gillespie (1979) who found that DNA fragments adsorbed to powdered flint glass in the presence of saturated NaI. Later work showed other nucleic acids adsorbed to glass in the presence of other chaotropes, including:

- DNA plasmids (Marko et al., 1982)
- Single-stranded phage nucleic acids (Kristensen et al., 1987; Zimmermann et al., 1989)
- Genomic DNA (Yamada et al., 1990; Zeillenger et al., 1993)
- Total RNA (Yamada et al., 1990)
- Nucleic acids from clinical samples (Boom et al., 1990)

Different types of nucleic acid adsorb more or less tightly to glass depending on the ionic strength and the pH of the surrounding solution. A low salt buffer or water is always used to elute the nucleic acid from the glass. In each kit, this method is optimized to prepare a particular type of nucleic acid.

Overview of the procedure

All the High Pure kits use glass fibre fleece immobilized in a special plastic Filter Tube. The glass fleece filter:

- Adsorbs only nucleic acid, ensuring separation of the target molecules from a complex biological mixture of proteins, sugars, lipids, and other components
- Can be inserted into a microcentrifuge tube and processed in a standard tabletop microcentrifuge
- Allows processing of 200 – 600 µl samples in a series of centrifugation steps
- Is specially constructed to ensure that contaminants suspended in the Wash Buffer are not retained by the filter or transferred to the eluted, purified nucleic acid

In each High Pure kit, the steps are basically the same and require only a few minutes. The nucleic acids prepared with the High Pure kits may be used directly in a variety of down-stream applications.

Substance added to serum	Highest concentration tested with no inhibition in PCR after High Pure purification
Citrate	30 mg/ml
EDTA	300 mg/ml
Heparin	30 U/ml
Hemoglobin	50 mg/ml

Performance of High Pure Nucleic Acid Purification in the removal of different anticoagulants or human hemoglobin. The table indicates the highest concentration tested which showed no inhibition.

Instead of glass fiber fleece (as in the High Pure kits), one can also use silica beads to adsorb DNA.

- The Agarose Gel DNA Extraction Kit starts with an agarose gel slice containing a DNA fragment, then solubilizes that gel to release the DNA into the starting solution

The kit uses steps similar to those of the High Pure kits to purify the DNA from the starting material. In each, the nucleic acid is adsorbed to silica in the presence of a chaotropic salt, pelleted by centrifugation (while adsorbed to the silica beads), washed extensively to remove contaminants, then released from the beads with a low salt buffer.

The DNA isolated with the kit is pure enough to be used directly in labeling, sequencing, cloning, and other procedures that require concentrated DNA.

References

- Boom, R., Sol, C.J.A., Salimans, M.M.M., Jansen, C.L., Wertheim-van Dillen, P.M.E. and van der Noordaa, J. (1990) *J. Clin. Microbiol.* **28**, 495 – 503
- Kristensen, T., Voss, H. and Ansoerge, W. (1987) *Nucleic Acids Res.* **15**, 5507 – 5516
- Marko, M.A., Chipperfield, R. and Birnboim, H.C. (1982) *Anal. Biochem.* **121**, 382
- Melzak, K.A., Sherwood, C.S., Turner, R.F.B. and Haynes, C.A. (1996) *J. Colloid Interface Sci. (USA)* **181**, 635 – 644
- Vogelstein, B. and Gillespie, D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 615 – 619
- Yamada, O., Matsumoto, T., Nakashima, M., Hagari, S., Kamahora, T., Ueyama, H., Kishi, Y., Uemura, H. and Kurimura, T. (1990) *J. Virol. Meth.* **27**, 203 – 210
- Zeillinger, R., Schneeberger, C., Speiser, P. and Kury, F. (1993) *Biotechniques* **14**, 202 – 203
- Zimmermann, J., Voss, H., Kristensen, T., Schwager, C., Stegemann, J., Erfle, H. and Ansoerge, W. (1989) *Methods Mol. Cell. Biol.* **1**, 29 – 34