

Overview of Solution-based Isolation

This chapter describes three specialized products. Each features a proprietary cell lysis method that is quicker and safer than standard lysis methods.

All of these products use methods that:

- Can be completed in minutes or hours, instead of days
- Can process multiple samples simultaneously
- Require less handling of potentially hazardous samples
- Minimize the use of toxic organic solvents

For a quick overview of each 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
DNA Isolation Kit for Mammalian Blood	Genomic DNA from larger volumes (1 – 10 ml) of mammalian whole blood	140
DNA Isolation Kit for Cells and Tissues	Genomic DNA from tissues, cultured cells, bacteria, yeast and mouse tail	148
TriPure Isolation Reagent	Total RNA, DNA, and protein (simultaneously) from cultured cells, blood, tissue, plants, yeast, and bacteria	156

DNA Isolation Kit for Mammalian Blood

Successful genomic Southern hybridizations and long-template PCR start with high quality purified DNA. Traditionally, preparing such DNA from blood required removal of hemoglobin by labor intensive methods such as density gradients and removal of protein and lipids with hazardous solvents such as phenol and chloroform (Sambrook et al., 1989).

The DNA Isolation Kit for Mammalian Blood provides an alternate approach for the isolation of DNA from 1 – 10 ml mammalian whole blood, buffy coat, or lymphocytes. The kit uses a special lysis reagent to selectively lyse erythrocytes. After all erythrocyte components are removed, leukocyte DNA can be isolated free of interfering hemoglobin.

The kit procedure requires approx. 90 min, and can easily process multiple samples. No organic extractions or column purifications are required, yet the isolated DNA is free of both protein and RNA.

The isolated DNA can be used in any application requiring genomic DNA, including long-template PCR and genomic Southern hybridizations.

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DNA Isolation Kit for Cells and Tissues

The kit offers a convenient, rapid method for the large scale isolation of intact, high molecular weight, genomic DNA from tissues, cultured cells, *E. coli*, yeast, and mouse tails.

The amount of genomic DNA recovered with the DNA Isolation Kit for Cells and Tissues is significantly higher than that obtained using alternative column-based methods in significantly less time. Starting material quantities ranging from 100 mg to 1 gram of tissue, or from 1×10^7 to 5×10^7 cultured cells, can be analyzed with the kit. Within 2 h plus resuspension time (for tissue samples), the kit yields pure genomic DNA suitable for amplification of long fragments by standard PCR, restriction enzyme digestion, and Southern blotting.

TriPure Isolation Reagent

Analysis of gene expression requires clean, intact RNA templates. Isolation of intact RNA is complicated primarily by a host of stable cellular ribonucleases. To minimize RNase activity, RNA isolation procedures typically begin with cell or tissue lysis in a strongly denaturing environment. The RNA is then separated from other cellular components via multiple phenol/chloroform extractions or time-consuming CsCl step gradients.

A simpler alternative, the TriPure Isolation Reagent, offers a rapid RNA isolation procedure that can easily process multiple samples and produces 30 – 150% more RNA than other purification methods. Briefly, the reagent causes RNA, DNA, and protein separate into different organic phases, from which each can be purified by a series of alcohol precipitations (Chomczynski, 1993).

Thus, the TriPure Isolation Reagent allows the simultaneous isolation of RNA, DNA, and protein from the same sample. The isolated total RNA is suitable for Northern blots, RT-PCR, poly(A⁺) fractionation, *in vitro* translation, or RNase protection assays. The isolated RNA-free DNA may be used for PCR, restriction digest, or Southern blots. The denatured protein may be analyzed on a Western blot.

References

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- Erlich, H.A., ed. (1989) *PCR Technology: Principles and Applications for DNA Amplification*, p. 31 – 38, Stockton Press, New York
- Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, vol. 2, p. 9.14 – 9.19, Cold Spring Harbor (N.Y.) Laboratory Press

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