SurePrep™ TrueTotal™ RNA Purification Kit – FAQ

Frequently Asked Questions:
(Please press ctrl-click on questions to see the answers)

Where can I find more information about the Fisher BioReagents RNA Purification Kits?

All relevant manuals, FAQs and ordering information can be found on the web at www.fishersci.com/RNAkits . Please contact your local Fisher Scientific Customer Service representative for any other questions you may have.

What sample types can be used with this kit?
Fisher BioReagents' SurePrep™ TrueTotal™ RNA Purification Kit can be used to isolate high-quality RNA from:

- Cultured cells
- Tissue samples
- Blood
- Bacteria
- Yeast/Fungi
- Plant cells and tissue

What sizes of RNA can be isolated using this kit?

All sizes of RNA can be isolated using this kit, from large mRNA and ribosomal RNA down to microRNA and small interfering RNA. Fisher BioReagents' purification technology does not preferentially isolate or exclude any particular sizes of RNA. Furthermore, this is accomplished without the use of phenol.

What is the advantage of having a phenol-free protocol?

Phenol is hazardous and can have an inhibitory effect on many downstream applications. Isolating truly total RNA in the absence of phenol is a major milestone in RNA isolation technology.

Also, the phase-separation step and the precipitation step in phenol-based protocols could result in significant sample loss. This could also lead to inconsistency in isolation. Phenol-free and column-based protocols allow more consistent RNA isolation which is very critical for studies such as expression analysis.

Does the kit remove genomic DNA?

Genomic DNA is removed to a very high degree. If complete DNA removal is required an optional on-column DNAse treatment protocol is provided within the manual.

What downstream applications have been tested using RNA isolated with this kit?

RNA isolated using Fisher BioReagents' RNA Purification Kits is highly suited to all downstream applications including:

- RT-PCR
- qRT-PCR
- miRNA amplification and quantification

- Northern blotting
- Microarrays

How do you assess the quality/integrity of the purified RNA?

The integrity of the RNA can be checked visually by running the RNA on a denaturing agarose gel. The main ribosomal RNA species should be present as clear, distinct bands. Alternatively, the RNA can be run on an Agilent ® BioAnalyzer according to the manufacturer's instructions. The BioAnalyzer will be able to give the values for the ratios of rRNA species, which can be compared to literature values in order to determine the integrity of the RNA. Using the BioAnalzyer, the RIN value (RNA Integrity Number) can be determined. On a scale of 1 to 10, RNA purified using Fisher BioReagents' kits routinely score in the 9.9 to 10.0 range.

What is the minimum amount of cells that I can isolate RNA from using Fisher BioReagents' SurePrep TrueTotal RNA Kit?

RNA has been isolated and detected from as little as a single animal cell (293 HEK cell) using this kit. The amount of cells that will yield the desired amount of final RNA depends on factors such as the specific RNA expression level of your sample and the type of down stream applications you intend to use. To determine the number of cells to use for your specific application, you may need to optimize the amount of starting material by running a series of dilutions of your control tissue/cell line before you purify your valuable sample.

Can the columns in the kit be re-used?

The columns are designed for single use only.

What is the maximum sample volume that I can load onto the column?

Quantities larger than 600 μL is not recommended. Please consult the product manual on the maximum number of cells/ starting material to use, as the total number of cells in the starting material is the limiting factor for the column capacity. If the density of your sample is very low it may be necessary to repeat the sample loading step. The maximum volume of the column is 700μL.

What is the typical yield of RNA that I can expect to purify using this kit?

The average yield of RNA obtained will depend on the sample type being used, the amount of starting material, the species, the growth conditions and the developmental stage of the sample. For example, 1×10^6 HeLa cells will generally yield about 15 µg of RNA, while 1×10^9 E. coli cells will yield up to 50 µg of RNA.

Can the columns be used in a vacuum processing?

The method has not been developed yet.

Can equipment other than mortar and pestle be used to prepare the samples? What other equipment can be used for tissue homogenization?

A hand-held microfuge homogenizer or mini bead beater is compatible to the protocol. Please operate the homogenizer under controlled conditions (e.g. temperature) to ensure the collection of quality RNA from the biological sample.

Can tissues stored in RNA stabilizing agents such as RNAlater be used?

Tissues stored in RNAlater are still compatible to the kit

Is there a convenient stop point during the protocol where a user may store samples for later processing?

While the column-based purification procedure is very short, it may take a lot of time to lyse multiple samples (for example, processing multiple mammalian tissue samples that require homogenization). The Lysis Solution provided contains a strong denaturant that inhibits RNase activity. Samples lysed in Lysis Solution may be stored at 4^{o}C for short term (days) or -80^{o}C for long term (months) before column purification.

Can β-mercaptoethanol be omitted?

β-mercaptoethanol helps inactivate RNases and hence is highly recommended, particularly for use with tissues that have a high RNase content.