DNase I Set (RNase-free)

#GKC01.0100 (100 preps)
(FOR RESEARCH ONLY)

Product: DNA contamination in final RNA preparations may interfere with several downstream applications such as gene expression analysis. Incubation with the RNase-free DNase I set ensures complete DNA removal from RNA solutions, as this enzyme hydrolyzes phosphodiester bonds of dsDNA, ssDNA, chromatin, and DNA-RNA hybrid molecules, without compromising RNA yield and integrity. This set can be used for both DNase digestion in solution as well as "in-column" digestion in combination with GRiSP's GRS total RNA Purification Kits.

Applications: DNA removal from RNA samples

Content: #GKC01.0100 contains 0.55 ml DNase I at a concentration of 2KU/µl and is supplied with an optimized reaction buffer and a stop solution.

Component	GKC01.0100
DNase I (2KU/µl)	0.55 ml
DNase I Reaction Buffer (10x)	5 ml
Stop Solution (20mM EGTA pH8.0)	100 µl

Properties: Free of RNases.
Specific Activity: >2KU/µl. (One Kunitz Unit (1 KU) is defined as the amount that is required to cause an increase in A260nm of 0.001/min/ml at 25ºC, using highly polymerized DNA as substrate).

QC: Absence of ribonuclease activity was confirmed by appropriate assay.

Storage: All components should be stored at -20ºC.

Prior to us

1. The DNase I Reaction Buffer may cause abnormal migration or even smearing of RNA on gels, so it may seem that RNA integrity has been compromised, however, this is not the case. If desired, one can analyze the purified RNA by gel electrophoresis, however, for that one should repurify RNA after step 2 using GRiSP´s GRS Pure RNA Kit, or equivalent, instead of stopping the reaction with Stop Solution.

2. This DNase I set can also be used for "in-column" digestion during the purification of RNA using one of GRiSP´s GRS Total RNA Purification Kits. The corresponding protocol is an integrated part of the protocol of these kits, which can be downloaded from our website.

Usage:

Typically, RNA is purified from samples using a column-based kit and eluted in the final step with 20-100µl of RNase-free water. This protocol is for the removal of all residual DNA following RNA elution.

1. Combine, in a RNase-free tube, 1µl - 40µl of eluted RNA with
   a. 0.5 µl of DNase I per µg of RNA and
   b. 5 µl of DNase I Reaction Buffer (10X)
   and add RNase-free water to a final reaction volume of 50µl

2. Mix by pipetting gently (do not vortex) and then incubate at 37°C for 20-30 minutes.

3. Stop the reaction by adding 1 µl of Stop Solution and incubate at 65°C for 10 minutes. Use purified RNA immediately for downstream application or store at -70°C.
4. If desired, DNase I can **be removed from the reaction mixture by standard phenol extraction.**