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Certificate of Inspection for DNase Activity

Purpose: The purpose of this procedure is to test and document that tips and tubes, manufactured by National Scientific Supply, are free of DNase activity.

Summary: Random lots of tips and tubes are analyzed for the presence of DNase activity. This is done by washing the tips and tubes with a ddH₂O / Magnesium Chloride PCR buffer. A DNase-Free 1kb DNA ladder is added to a representative sample of the wash, and the entire mixture is incubated. After incubation, the sample mixture is examined by gel electrophoresis.

Procedure:

- 1) A running gel 1xTAE buffer and 2% agarose gel is prepared.
- 2) Using sterile technique, tips and tubes to be tested are washed with 5ml of a prepared ddH₂O / Magnesium Chloride PCR buffer. In order to detect marginal nuclease contamination, each wash consists of 3 repetitions.
- 3) DNase-Free pipet tips are used to extract a 2 μ l sample of the PCR buffer wash.
- 4) 10 μ l of a 1kb DNA ladder and 2 μ l PCR buffer wash are added into DNase-Free tubes.
- 5) This sample mixture is briefly centrifuged and then incubated at 37°C for 3 hours.
Do Not Vortex.
- 6) Following incubation, 1 μ l of a DNase-Free gel loading dye is added to each of the sample mixtures, and then briefly centrifuged again.
- 7) A prepared 12 μ l 1kb DNA ladder is used for the control.
- 8) The 12 μ l 1kb DNA ladder control, several 13 μ l sample mixtures, and 13 μ l of the intentionally contaminated sample, are loaded into a DNase-Free 2% agarose gel.
- 9) The gel is run at 70V for 1.5 hours, in a 1xTAE buffer containing 4 μ l of ethidium bromide, then removed from the gel apparatus and photographed.



Lane #1 contains a 1kb DNA ladder control.
Lane #2, Lane #4, and Lane #5 contain sample mixtures being tested for DNase activity.
Lane #3 contains a positive control generated from the intentional contamination of the sample.

Interpreting the gel results: If DNase is present in the sample mixture, there will be a very noticeable smearing in the sample lanes, and band intensities will decrease. Lane #1 shows the 1kb DNA sizing control ladder for gel orientation and size standards. Lane #2, Lane #4, and Lane #5 lack smearing, indicating the tips and tubes tested are DNase-Free. Lane #3 contains the expected smearing from the intentionally contaminated sample.

Conclusion: Random samples of National Scientific Supply's tips and tubes are DNase-Free.