CERTIFICATE OF STERILIZATION

INSPECTION FOR DNA, RNA, DNase, RNase, ATP AND ENDOTOXINS

pipetman® TIPS

DIAMOND Pure Performance Tips!

STERILIZED PIPETMAN DIAMOND TIPS

The manufacturing and sterilization process Gilson follows are certified to produce sterilized PIPETMAN DIAMOND Tips **free of detectable DNA, RNA, DNase, RNase, ATP and Endotoxins** (Pyrogens). An independent accredited laboratory [NF EN ISO/CEI 17025 (COFRAC)] periodically audits these parameters.

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The manufacturing lot of tips listed below has been Gamma irradiated. The Bioburden analysis is carried out quarterly on a sample of tips drawn from current production in order to ensure a SAL of 10^{-6} (ISO 11137).

BIO-MOLECULE DETECTION PROTOCOLS

For all tests, disposable tips are rinsed with sterile DNase-RNase free water called "liquid extract". Negative controls, positive and extract controls are made to validate all steps of the different assays.

• DNA detection < 2 pg

Six products are rinsed 20 times with sterile DNase-RNase free water. Then, liquid extract (10 µL) is exposed to PCR reaction reagents containing specific primers of human DNA. Amplicons are obtained after 35 cycles of amplification, and are analysed by electrophoresis on a 1% agarose gel coloured by Syber Green I. None specific signal of 200 pb must be observed for the samples tested. Positive controls (7 known amounts of genomic human DNA: 50 ng, 10 ng, 500 pg, 50 pg, 10 pg, 5 pg and 2 pg) and a negative control (10 µL of DNase-RNase free water) are included in the assay.

• RNA detection < 2 µg/mL

The RNeasy® micro kit (Qiagen) is used to obtain liquid extracts. Six products are rinsed 20 times with RTL buffer (buffer provided in the kit) before performing the extraction procedure recommended by the manufacturer. The mixture is then exposed to UV light and the intensity of the samples' signals is compared with a negative control (DNase-RNase free water) and with RNA standards (2, 4, 6, 10 µg/mL).

• DNase detection < 5.2 x 10^{-6} Kunitz units

Six products are rinsed 20 times with sterile DNase-RNase free water. 10 µL of liquid extract are incubated 10 minutes at room temperature (20-21°C) with variable concentrations of DNA ladder (10 ng, 50 ng and 100 ng). Positive controls (10 ng, 50 ng and 100 ng of DNA ladder) are incubated, in the same conditions, with 5.2 x 10^{-6} Kunitz units of DNase I. Positive and negative controls (10 µL of DNase-RNase free water) are included in each test. Samples are analysed by electrophoresis (1% agarose gel containing ethidium bromide). The intensity of samples' signals is compared with negative and positive controls. The degradation of DNA ladder indicates the presence of DNase in the liquid extract.

• RNase detection < 2.9 x 10^{-9} Kunitz units

Six products are rinsed 20 times with sterile DNase-RNase free water. 10 µL of liquid extract are incubated 10 minutes at 25°C with 350 ng of RNA ladder. Positive control (350 ng of RNA ladder) are incubated, in the same conditions, with 17.5 10^{-9} Kunitz units of RNase A. Positve and negative controls (10 µL of DNase-RNase free water) are included in each test. Samples are analysed by electrophoresis (1.5% agarose gel ethidium bromide). The intensity's signals of samples are compared with negative and positive controls. The degradation of RNA ladder indicates the presence of RNase in the liquid extract.

• ATP detection < 5x10^{-18} mol/L

Six products are rinsed 20 times with sterile DNase-RNase free water. 100 µL of liquid extract are incubated 10 minutes at 25°C in presence of 100 µL of recombinant luciferase. A negative control (DNase-RNase free water), positive control (ATP 5.10^{-5} mol/L), extract control and ATP standard curve (5 x 10^{-18}, 5 x 10^{-17}, 5 x 10^{-16}, 5 x 10^{-15}, 5 x 10^{-14}, 5 x 10^{-13} mol/L) are incubated in the same conditions. The luminescence is recorded. There is a linear relationship between the luminescence signal and the quantity of ATP.

• Endotoxin detection < 0.005EU/mL

Gilson sterilized PIPETMAN DIAMOND Tips certified non-pyrogenic have been tested for bacterial endotoxins. Samples selected at random were tested and validated using the LAL kinetic chromogenic method D with < 0.005EU/mL sensitivity. *European Pharmacopoeia section 2.6.14 Methodology for bacterial endotoxin testing.*

NB:** Additional protocol to test the **limit of detection < 0.001EU/mL was conclusive.

Product Designation	Catalog Number	Lot Number	Expiration of Sterilization
DF30ST BLISTER REFILL	F172303	UBGH3/00020	⧖ 10/01/2018

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OUR PIPETTES CONFORM TO ISO 8655

GILSON®