Certificate of Sterilization

Inspection for DNA, RNA, DNase, RNase, ATP, and Endotoxins

pipetman® DIAMOND 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.

CERTIFICATE OF STERILIZATION

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.

- **Human DNA Detection < 2 pg**

Preparation of sample with sterile and pyrogen free water. Extraction of DNA with a commercial kit. An aliquot of liquid extract is exposed to PCR reaction reagents containing primers (to amplify the β-actin housekeeping gene). A range of human DNA (2, 5, 10, 20 and 50 pg) is also amplified and used as positive control. Amplicons are obtained after cycles of amplification, analyzed by electrophoresis on a 2% agarose gel into TBE 0,5X buffer. No DNA have to be detected in the sample.

- **RNA Detection < 1 pg**

Preparation of sample with sterile and pyrogen free water. Extraction, purification and RNA concentration are performing with a kit, and RNA converted to cDNA with an other kit, procedure recommended by the manufacturer. The cDNA is exposed to PCR reaction reagents containing primers (to amplify the β-actin housekeeping gene). A range of human RNA (1, 2, 4, 8 and 16 pg) is also amplified and used as positive control. Amplicons are obtained after cycles of amplification, analyzed by electrophoresis on a 2% agarose gel into TBE 0,5X buffer. No RNA have to be detected in the sample.

- **DNase Detection < 12,5 pg**

Preparation of sample with sterile and pyrogen free water. 10 µL of liquid extract are incubated 2 hours at 37°C with variable concentrations of 1Kb plus DNA ladder (0, 10, 50 and 100 ng). Positive controls (0, 10, 50 and 100 ng of DNA + DNase I: 12,5 pg) and negative controls (0, 10, 50 and 100 ng of DNA) are incubated in the same conditions. Samples are analyzed by electrophoresis (2% agarose gel into TBE 0,5X buffer). The intensity of samples signals is compared with negative and positive controls. The degradation of DNA indicates the presence of DNase in the liquid extract. No degradation have to be observed, the intensity of negative control and the sample have to be identically.

- **RNase Detection < 0,25 ng**

Preparation of sample with sterile and pyrogen free water. 10 µL of liquid extract are incubated 10' at room temperature (20-25°C) with 350 ng of RNA (0,1 – 2Kb RNA ladder). Positive control (350 ng of RNA + 0,25 ng of RNase A) and negative control (350 ng of RNA) are incubated in the same conditions. Samples are analyzed by electrophoresis (2% agarose gel into TBE 0,5X buffer). The intensity of samples signals is compared with negative and positive controls. The degradation of RNA indicates the presence of RNase in the liquid extract. No degradation have to be observed, the intensity of negative control and the sample have to be identically.

- **ATP Detection < $1x10^{-18}$ mol/L**

Preparation of sample with sterile and ATP free water. Evaluation of ATP by luminescence (enzymatic reaction of luciferase - commercial kit). The intensity of the samples signals is compared with a negative control (DNase-RNase free water) and with ATP standards curve. The intensity of samples signals is compared with negative and positive controls curve. No ATP have to be detected.

- **Endotoxin Detection < 0,005 EU/mL**

Gilson sterilizied 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 < 0.005 EU/mL sensitivity. *European Pharmacopoeia section 2.6.14 methodology for bacterial endotoxin testing.*

PRODUCT DESIGNATION	CATALOG NUMBER	LOT NUMBER	EXPIRATION OF STERILIZATION
D200ST TIPACK	F171301	ELAD5/02660	2030-09

www.gilson.com/ContactUs

ISO 8655 CERTIFIED

GILSON®