PCR Certification

These products have been tested and are certified free of DNase or RNase contamination, and from contamination with human genomic DNA.

DNase test procedure.

Sample tubes are pipetted with 60µL sterile PCR buffer, vortexed, then spun down. The buffer is transferred to a second tube. This procedure is repeated 14 times until the buffer has been exposed to all the tubes of the sample batch except one. 32µL from the 15th tube is transferred to the final tube then 8µL of 25mM Magnesium Chloride solution and 5µL of pBR322 DNA added. The tube is incubated at 37°C for two hours with three positive controls (spiked with DNase I).

The plasmid DNA is then analysed on a 1.5% agarose gel with the positive controls and a negative control (no incubation). The gel is stained with Ethidium Bromide.

A brighter band corresponding to the relaxed form of the plasmid indicates DNase contamination, where the plasmid DNA has been nicked by DNases. For samples to pass certification, the relative intensities of the supercoiled and relaxed plasmid DNA bands from solutions exposed to the batch samples must correspond to the negative control.

RNase test procedure.

Sample tubes are pipetted with 60µL sterile PCR Buffer, vortexed, then spun down. The buffer is transferred to a second tube. This procedure is repeated 14 times until the buffer has been exposed to all the tubes of the sample batch except one. 40µL from the 15th tube is then transferred to the final tube and 5µL of tRNA added. The tube is incubated at 37°C for two hours with three positive controls (spiked with RNase A).

The tRNA is then analysed on a 4% agarose gel with the positive controls and a negative control (no incubation). The gel is stained with Ethidium Bromide.

RNase contamination is indicated by a smear, where the tRNA has been degraded by RNases. For samples to pass certification, the tRNA band from solutions exposed to the batch samples must correspond to the negative control with no evidence of smearing.

Human DNA Contamination test procedure.

A PCR Master Mix is prepared containing Reaction Buffer IV, Taq DNA polymerase, dNTPs and primers L1-5' and L1-3'. These amplify a 294bp fragment present at more than 10^5 copies in human cells (J.M. Deragon et al, (1990). NAR, **18:** p.649). 50µl of the Master Mix is added to 5 positive control tubes containing known amounts of human DNA (32, 16, 8, 4, and 2pg) plus a negative control (gamma irradiated tube & cap with 50µl master mix). The sample tubes are pipetted with 60µl of Master Mix, vortexed, then spun down. The Master Mix is then transferred to a second tube. This is repeated 14 times until the Master Mix has been exposed to all the tubes in the batch except one. 50µl from the 15th tube is transferred to the final tube, then PCR is performed for 26 cycles in parallel with the positive and negative control(s). This procedure will detect 2pg of genomic DNA, equivalent to less than one human cell. Samples and controls are analysed on a 2% agarose gel stained with Ethidium Bromide. For the samples to pass certification, there should be no PCR product in the sample lanes.

PCR (polymerase chain reaction) is covered by U.S. Patents owned by Hoffmann-La Roche. Nothing in this certificate should be construed as an authorisation or implicit licence to practice PCR under any patents held by Hoffman-La Roche.