Microzone Ltd

Human cDNAOK!

Protocol:

- Mix together 7.5 µl of Human cDNAOK mix and 12.5 µl of MegaMix~Gold
- Add 1 - 5 µl cDNA (adjust volume with water if necessary)
- Overlay with mineral oil if necessary.
- Place in a Thermal Cycler

Cycling profile:

Initial denaturation step: 95°C for 3 mins

Then cycle 33 times:

Step 1: 95°C for 20 secs

Step 2: 59°C for 20 secs

Step 3: 72°C for 40 secs

After cycling, load 10 µl onto 1.75% agarose gel and electrophorese alongside a 100 bp DNA Ladder (not supplied). Make sure that the sample hasn't evaporated during cycling, as this will distort the results.

Interpretation of results:

Expected fragment sizes: 125 bp, 250, 375, 500 and 650 bp.

- **If all 5 fragments are observed the cDNA is more than likely to be okay**
- The 500 bp fragment is derived from an internal control and should always be present (even in negative controls). If not, PCR has failed and needs repeating
- If less than 5 fragments are observed the cDNA is likely to not be okay
- If only the control fragment is observed then the cDNA is likely to not be okay, not enough or not added
- Different band intensities can represent different levels of cDNA.

Store at -20°C

For Research Only