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# INSTRUCTIONS FOR USE

**Product Name: Fast and High-Fidelity PCR Mix (With Dye)** **Catalog # HF02-M29A**

**Residual genomic DNA removal:**

**Step 1.** In a centrifuge tube add the following reaction components:

| | |
|---|---|
| 2 µL (0.4 µM) | Forward Primer (10 µM) |
| 2 µL (0.4 µM) | Reverse Primer (10 µM) |
| X µL | Template |
| 25 µL (1x concentration) | Fast and High-Fidelity PCR Mix (with dye) |
| Up to 50 µL | $ddH_2O$ |

- The PCR Mix contains 2mM $Mg^{2+}$ and 200µM dNTPs.
- The recommended amount of cDNA template is 10-200ng, not more than 10% of the reaction volume (1-2.5 µL).
- The final primer concentration in the reaction can range from 0.2-1 µM (0.4µM is recommended).

**Step 2.** Run the following thermal cycling settings:

| | | | |
|---|---|---|---|
| Pre-denaturation | 98°C | 30 sec | 1 cycle |
| Denaturation | 98°C | 10 sec | 30-35 cycles |
| Annealing | 60°C | 5 sec | |
| Extension | 72°C | 5 sec/kb | |
| Final Extension | 72°C | 2 min | 1 cycle |

- The annealing temp can be optimized and set to a temp gradient. The time can be adjusted between 5-30sec. Too long annealing time can result in the amplification products being diffused on the gel.
- The extension time can be optimized up to 10 sec/kb.