

Detection of species specific milk (goat/sheep/cow) content in dairy

➤ KIT COMPONENTS

Species Specific Mitochondrial Cytochrome b Mastermix (store at -20°C)

Taq Polymerase (store at -20°C)

➤ PROCEDURE

Note:

Thaw kit reagents just before use. Mix thawed reagents thoroughly.

Do not vortex the enzyme-containing mastermix (working mastermix). Only thaw as many PCR mastermix tubes as are required (aliquots of 50 μl are appropriate).

1. Thaw PCR mastermix solutions on ice.

2. Set up mastermix and PCR components according to the table below:

Component	Volume per reaction (μl)
Mastermix	4
DNA template	X
Double-distilled water	15.85 – X
Taq Polymerase	0.15
Total Volume	20

3. Use appropriate volume of DNA template and double-distilled water. Use ~2 ng DNA template for each PCR reaction. (DNA concentration should have been determined spectrophotometrically after DNA extraction steps).

When all PCR reactions are set up, load all PCR tubes into the PCR thermal cycler and use the cycling conditions shown below:

1 Cycle: 95°C for 5 minutes
 35 Cycles: 95°C for 30 seconds
 60°C for 30 seconds
 72°C for 45 seconds
 1 Cycle: 72°C for 20 minutes

➤ DATA ANALYSIS AND INTERPRETATION

Expected product size: 336 bp (Sheep)

287 bp (Cow)

313 bp (Goat)