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## Duplex real-time PCR assay using SYBR Green to detect and quantify Malayan box turtle (*Cuora amboinensis*) materials in meatballs, burgers, frankfurters and traditional Chinese herbal jelly powder

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### FOOD ADDITIVES AND CONTAMINANTS PART A-CHEMISTRY ANALYSIS CONTROL EXPOSURE & RISK ASSESSMENT

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### Abstract

The Malayan box turtle (*Cuora amboinensis*) (MBT) is a vulnerable and protected species widely used in exotic foods and traditional medicines. Currently available polymerase chain reaction (PCR) assays to identify MBT lack automation and involve long targets which break down in processed or denatured tissue. This SYBR Green duplex real-time PCR assay has addressed this research gap for the first time through the combination of 120- and 141-bp targets from MBT and eukaryotes for the quantitative detection of MBT DNA in food chain and herbal medicinal preparations. This authentication ensures better security through automation, internal control and short targets that were stable under the processing treatments of foods and medicines. A melting curve clearly demonstrated two peaks at 74.63 +/- 0.22 and 78.40 +/- 0.31 degrees C for the MBT and eukaryotic products, respectively, under pure, admixed and commercial food matrices. Analysis of 125 reference samples reflected a target recovery of 93.25-153.00%, PCR efficiency of 99-100% and limit of detection of 0.001% under various matrices. The quantification limits were 0.00001, 0.00170 +/- 0.00012, 0.00228 +/- 0.00029, 0.00198 +/- 0.00036 and 0.00191 +/- 0.00043 ng DNA for the pure meat, binary mixtures, meatball, burger and frankfurter products, respectively. The assay was used to screen 100 commercial samples of traditional Chinese herbal jelly powder from eight different brands; 22% of them were found to be MBT-positive (5.37 +/- 0.50-7.00 +/- 0.34% w/w), which was reflected through the Ct values (26.37 +/- 0.32-28.90 +/- 0.42) and melting curves (74.63-78.65 +/- 0.22 degrees C) of the amplified MBT target (120 bp), confirming the speculation that MBT materials are widely used in Chinese herbal desserts, exotic dishes consumed with the hope of prolonging life and youth.

### Keywords

**Author Keywords:** [Malayan box turtle](#); [SYBR Green real-time PCR](#); [limits of detection and quantification](#); [protected species](#); [herbal jelly powder](#)

**KeyWords Plus:** [POLYMERASE-CHAIN-REACTION](#); [DEER CAPREOLUS-CAPREOLUS](#); [CYTOCHROME-B GENE](#); [MEAT-PRODUCTS](#); [SPECIES IDENTIFICATION](#); [POTENTIAL FRAUD](#); [SUITABLE METHOD](#); [FALLOW-DEER](#); [FOOD-CHAIN](#); [RED-DEER](#)

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