**Processing procedure**

If frozen - how and how quickly? : By liquid nitrogen flash freezer

**Sample**

Sample storage conditions and duration：1 days at -80℃

**Nucleic acid extraction**

Nucleic acid quantification:

Instrument and method:We used NanoDrop 2000 to evaluate nucleic acid quantification

Purity (A260/A280): From 1.9 to 2.1

**Reverse transcription**

Complete reaction conditions

Amount of RNA and reaction volume

Priming oligonucleotide (if using GSP) and concentration

Reverse transcriptase and concentration

Step1. Genomic DNA removal

Prepare the following mixture in an RNase free centrifuge tube

|  |  |
| --- | --- |
| RNase-free ddH2O | to 16 μl |
| 4 × gDNA wiper Mix | 4 μl |
| Oligo (dT) 23 VN (50 μM) | 1 μl |
| Random hexamers (50 ng/μl) | 1 μl |
| Total RNA or Poly A+ RNA | 10 pg - 1 μg |

Step2. Prepare the first strand cDNA synthesis reaction solution

Prepare the following mixture in an RNase free centrifuge tube:

|  |  |
| --- | --- |
| Mix of Step1 |  |
| 10× RT Mix |  |
| HiScript II Enzyme Mix |  |

Temperature and time

|  |  |
| --- | --- |
| 50°C | 15min |
| 85°C | 2min |

Storage conditions of cDNA: At -20℃

**qPCR TARGET INFORMATION**

Location of amplicon

Amplicon length：107bp

**qPCR oligonucleotides**Manufacturer of oligonucleotides：Sangon Biotech(Shanghai)Co.,Ltd

**qPCR protocol**

Manufacturer of plates/tubes and catalog number：LABSELECT MP-96-HS-0200 and LABSELECT SF-001-UC-25