Promega

Product Application

TNA extraction from feces using Maxwell® RSC instrument

TNA from pork, hen and cow feces was extracted using Maxwell® RSC blood kit

Kit: Maxwell® RSC blood kit (Cat. #AS1400)

Analyses: qPCR and RT-qPCR

Sample Type(s): Feces (pork, hen and cow)

Input: 80 to 150 mg of feces

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

Further information can be found in Technical Manual #TM419, available at: www.promega.com/protocols

Materials Required:

- Feces samples
- Maxwell® RSC blood kit (Cat. #AS1400)
- Maxwell® RSC instrument (Cat.#AS4500)
- Thermobloc
- Centrifuge
- Optional: Bead beater (e.g: FastPrep 24 from MP biomedical)
  Bead (e.g: lysing matrix B from MP biomedical)

Protocol:

- Weight 80 to 150 mg of feces
- Add 600µl of lysis buffer
  - Optional: Add in a 2ml tube with bead and apply bead beating treatment as recommended by the manufacturer. (e.g: lysing matrix B from MP biomedical with Fastprep®24 instrument). This step will improve the lysis of bacteria
- Vortex vigorously
  - Optional: Incubate 5min at 95°C (this step would improve virus lysis and so improve sensitivity of detection)
- Centrifuge at 13000rpm for 5min
- Take 300µl of supernatant in a new tube
- Add 30µl Proteinase K
- Incubate 20 min at 56°C
- Centrifuge 2 min at 13000rpm
- Add 300µl on well 1 of the Maxwell cartridge
- Add 300µl of nuclease free water on well 1 of the cartridge
- Run the Maxwell RSC blood protocol
- Elute in 50µl

Results:

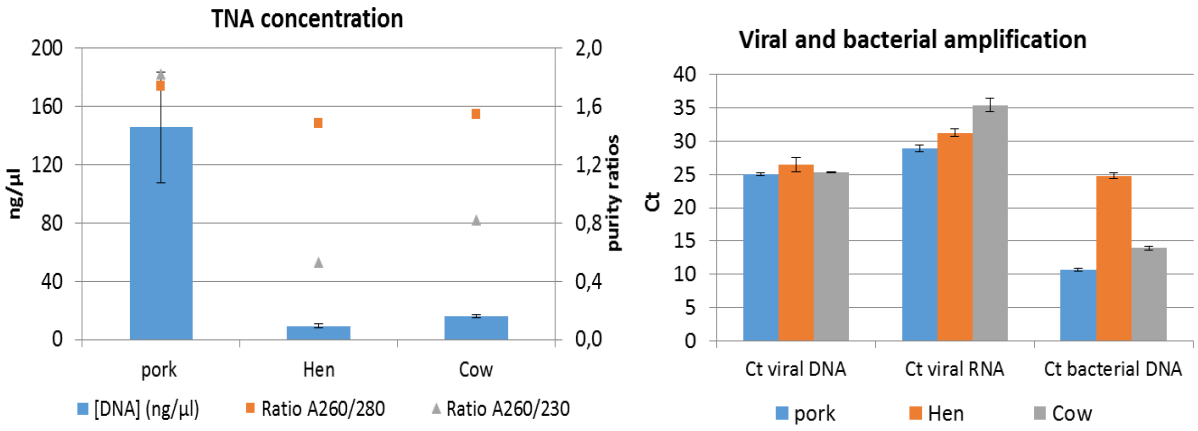

Figure 1 : Right: DNA concentration and purity ratios of TNA extracted from pork, hen and cow feces measured using Nanovue (n=3). Left: Lambda DNA virus, MS2 RNA virus and bacteria detection by qPCR or RT-qPCR on TNA extracted from pork, hen and cow feces spikes with Lambda DNA virus and MS2 RNA virus.

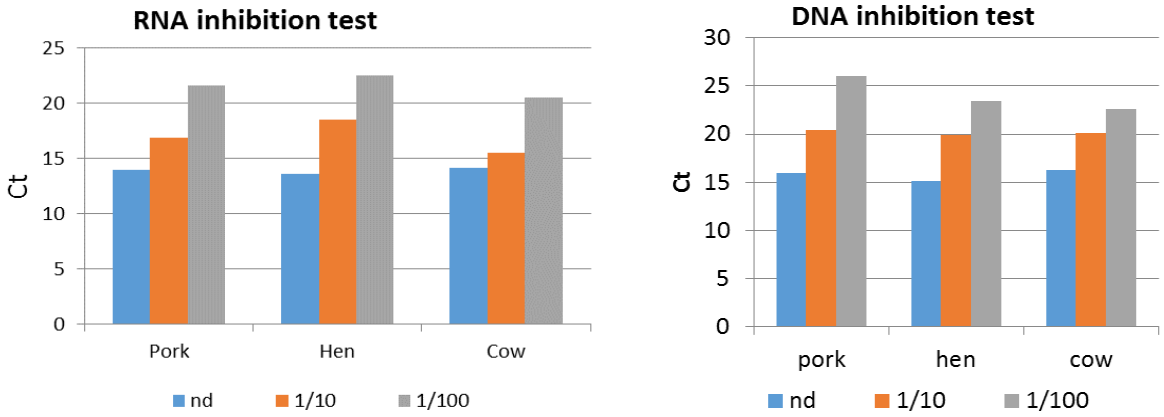

Figure 2 : Right: MS2 RNA amplification on 2µl of TNA non diluted (nd), diluted by 1:10 or by 1/100 extracted from pork, hen and cow feces spikes with lambda DNA and MS2 virus RNA. Amplification was performed with GoTaq® 2-step RT-qPCR system (A6010). Left: MS2 DNA amplification on 2µl of TNA non diluted (nd), diluted by 1:10 or by 1:100 extracted from pork, hen and cow feces spikes with lambda DNA and MS2 virus RNA. Amplification was performed with GoTaq® probe qPCR master mix (A6101).