



**9. PROCEDURE**

**9.1 Preparation of Standard Curve dilution series**

Always wear gloves for all PCR procedures.

1. Thaw the DNA Standard (10%). Mix thoroughly and centrifuge briefly
2. Prepare different DNA concentrations, according to your preferences. Dilute the Standard DNA in water, PCR grade or Tris-buffered solution (TE). Table 2 shows an example:

**Table 2** - Dilutions of Standard DNA

Dilution series	Dilution step	%
1	Standard DNA (10%)	10 %
2	25 µL TE + 25 µL Standard DNA (10%)	5 %
3	90 µL TE + 10 µL Standard DNA (10%)	1 %

**9.2 PCR preparation**

**A – PCR mix**

For reliable quantitative results, it is recommended to apply the DNA Standard SUPREME Swine in combination with the SUPREME Real Time Detection Kit Swine (BIOPSFS-0007 or BIOPSFS-sp0007). Prepare the real-time PCR reaction mix, as recommended by the manufacturer, then:

3. Add 15 µL of the real-time PCR reaction mix into each tube/strip.
4. Add 5 µL of each DNA Standard (Table 2). Triplicate reactions are recommended.
5. Add 5 µL of DNA sample (10 ng/µL).
6. Close the tubes/strips, and centrifuge briefly.
7. Place the reactions into the Real Time PCR instrument.

**10. DATA ANALYSIS**

Compare the Ct's values of each DNA Standard with the samples.



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BPMP is certified ISO 9001:2015

**1. BACKGROUND INFORMATION**

According to the European Commission Directive 2002/86/EC, food ingredients have to be declared. Species authenticity is highly relevant to consumers for economic, medical, cultural, and religious reasons. Examples like fraudulent substitution of expensive species with cheaper meats, inclusion of meat in vegetarian products, and allergens in food products highlight the importance of this issue. Additionally, meat species must be disclosed on the label with both commercial and scientific denominations to verify the meat's origin and traceability and to analyze the quality control of handling and cleaning processes of production lines.

**2. INTENDED USE**

The DNA Standard SUPREME Swine is designed to perform a comparative quantitation analysis of swine DNA in food and feedstuff samples by Real Time PCR. For reliable quantitative results it is recommended to apply the DNA Standard SUPREME Swine in combination with the SUPREME Real Time Detection Kit Swine (BIOPSFS-0007 or BIOPSFS-sp0007).

**3. TRADEMARKS AND DISCLAIMER**

Registered names, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law. BPMP kit handbooks and user manuals can be requested from BPMP or your local distributor.

**4. LIMITED LICENSE AGREEMENT**

Use of this product signifies the agreement of the following terms: The kit must be used solely in accordance with the respective Instructions for Use. BPMP grants no license under any of its intellectual property to use or incorporate the enclosed components of this Kit with any components not included within this kit except as described in the Instructions for Use. This kit and its components are licensed for one-time use and may not be reused, refurbished, or resold.

**5. QUALITY CONTROL**

In accordance with BPMP's ISO 9001, each lot of the kit is tested against predetermined specifications to ensure consistent product quality.

**6. WARNINGS AND PRECAUTIONS**

Molecular Biology procedures, such as RNA extractions and PCR amplification, require qualified staff to prevent the risk of erroneous results, especially due to sample contamination or degradation of the nucleic acids contained in the samples. It is strongly recommended to have dedicated areas, materials and equipment for the RNA extraction, preparation of the PCR and post-PCR procedures. Workflow in the laboratory must proceed in a unidirectional manner, beginning in the Extraction Area and moving to the Amplification and Detection Area.

- The user should always pay attention to the following:
- Read all the instructions provided before running the assay.
- Do not mix reagents from different batches.
- Wear proper PPE, including disposable gloves and laboratory coats.
- Store and extract positive material separately from all other reagents.

**7. CONTENTS AND STORAGE CONDITIONS**

The kit should be stored between -20°C and 5°C. Repeated thawing and freezing (>5) should be avoided, as this may reduce the sensitivity. Considerate to freeze in aliquots to maintain the performance of the assay.

CONTENTS	UNITS	COMPOSITION
DNA Standard (10%) (white cap)	1 tube (1 x 700 µl)	DNA standard and storage buffer

**8. MATERIAL REQUIRED AND NOT SUPPLIED**

- SUPREME Real Time Detection Kit Swine (BIOPSFS-0007 or BIOPSFS-sp0007)