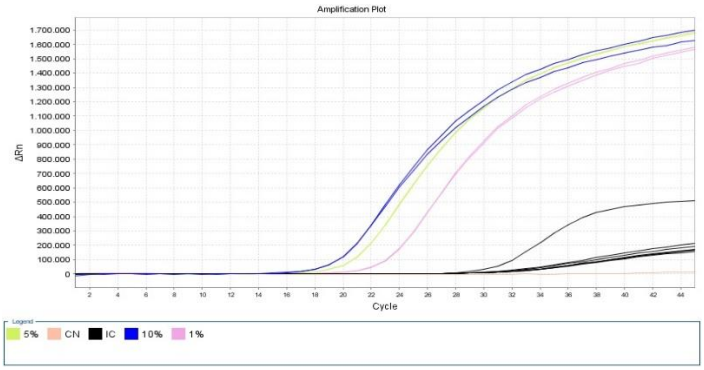

9. PROCEDURE

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:

Tabel 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 Alaska pollock in combination with the SUPREME Real Time Detection Kit Alaska pollock (BIOPSFS-058 or BIOPSFS-sp058). 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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BIOPREMIER

SUPREME
DNA STANDARD

ALASKA POLLOCK

Ref: BIOPDNA-058

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 fishes, inclusion of fish in vegetarian products, and allergens in food products highlight the importance of this issue. Additionally, fish 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 Alaska pollock is designed to perform a comparative quantitation analysis of Alaska pollock DNA in food and feedstuff samples by Real Time PCR. For reliable quantitative results it is recommended to apply the DNA Standard SUPREME Alaska pollock in combination with the SUPREME Real Time Detection Kit Alaska pollock (BIOPSFS-058 or BIOPSFS-sp058).

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. BPMR kit handbooks and user manuals can be requested from BPMR 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. BPMR 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 BPMR'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 Alaska pollock (BIOPSFS-058 or BIOPSFS-sp058)