Extracta DBS

Cat No: 95171-010 Size: 10 mL **Store at 2°C to 8°C**
95171-500 500 mL

Description

Extracta DBS is a ready-to-use DNA extraction reagent for rapid and efficient recovery of PCR-ready DNA from dried blood spots (DBS) on Guthrie cards or Whatman 903 filter paper. This patented single-solution process produces DNA that is suitable for a variety of downstream applications including real-time qPCR or template generation for Next Generation Sequencing (NGS) or Sanger sequencing (1-3). DNA eluates are substantially free of PCR inhibitors and compatible with a variety of end-point PCR or real-time qPCR reagents. Application of Extracta DBS with PerfeCta® qPCR ToughMix® or PerfeCta® MultiPlex qPCR ToughMix enables accurate and reproducible quantification of DNA sequences in blood using TaqMan® hydrolysis probe real-time qPCR.

Components

Extracta DBS	1X concentrated, ready-to-use, proprietary DNA extraction reagent

Storage and Stability

Extracta DBS is stable for up to 1 year at 2°C to 8°C.
For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form

Recommended Protocol for DNA Isolation from Dried Blood Spots (DBS)

1. Place a single 3.2 mm punch from DBS specimen in each PCR tube or well of a PCR plate.
2. Add 100 µL of Extracta DBS reagent and rinse the DBS punch by gentle vortexing or pipetting up and down.
3. Cap the tubes (or seal the plate) and centrifuge at ~3,500 rpm for 5 minutes.
4. Using a pipettor, remove as much liquid and debris as possible and discard the rinse into 10% bleach or in accordance with your institution's safety policies for processing dried blood spot specimens.
5. Add 50 µL of fresh Extracta DBS reagent to each rinsed DBS specimen.
6. Cap the tubes (or seal the plate) and then briefly centrifuge (10-30s) at ~3,500 rpm to ensure that the punch is completely submerged in the reagent.
7. Place the tubes or plate in a thermal cycler with a heated lid and incubate at 95°C for 20 minutes, then hold at 4-8°C.
8. Remove the tubes or plate from the thermal cycler and use up to 5 µL of extracted DNA as template in each 20-25-µL PCR.

Note: it is normal for the extracted DNA solution to have a pink color.

Extracted DNA should be used immediately in down-stream amplification procedures. It may be refrigerated at 2-8°C for short term storage before PCR amplification. The effect of prolonged storage on specific applications may vary.

Reagents and Equipment Required but not Provided

- Microcentrifuge tubes (0.5 mL or 1.5 mL), PCR tubes (0.2 mL) or multiwell plates
- P100 / P200 pipettor or multichannel pipettor
- PCR reagents
- Thermal cycler

Related Products

PerfeCTa qPCR ToughMix, 250R Cat. No. 95112-250,
PerfeCTa qPCR ToughMix, 5000R, Cat. No. 95112-05K,
PerfeCTa qPCR ToughMix, 1250R, Cat. No. 95112-012

PerfeCTa qPCR ToughMix ROX, 250R, Cat. No. 95113-250,
PerfeCTa qPCR ToughMix ROX, 5000R, Cat. No.95113-05K,
PerfeCTa qPCR ToughMix ROX, 1250R, Cat. No. 95113-012

PerfeCTa qPCR ToughMix L-ROX, 250R, Cat. No.95114-250,
PerfeCTa qPCR ToughMix L-ROX, 5000R, Cat. No.95114-05K,
PerfeCTa qPCR ToughMix L-ROX, 1250R, Cat. No.95114-012

PerfeCTa Multiplex qPCR ToughMix, 250R, Cat. No.95147-250,
PerfeCTa Multiplex qPCR ToughMix, 1000R, Cat. No.95147-01K,
PerfeCTa Multiplex qPCR ToughMix, 5000R, Cat. No.95147-05K

PerfeCTa Multiplex qPCR ToughMix, ROX 250R, Cat. No. 95148-250,
PerfeCTa Multiplex qPCR ToughMix, ROX 1000R, Cat. No. 95148-01K,
PerfeCTa Multiplex qPCR ToughMix, ROX 5000R, Cat. No. 95148-05K

PerfeCTa Multiplex qPCR ToughMix, L-ROX 250R, Cat. No. 95149-250,
PerfeCTa Multiplex qPCR ToughMix, L-ROX 1000R, Cat. No.95149-01K,
PerfeCTa Multiplex qPCR ToughMix, L-ROX 5000R, Cat. No.95149-05K

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References

- Baker et al. 2016. Improving newborn screening for cystic fibrosis using next-generation sequencing technology: a technical feasibility study. Genet Med. 18(3):231-238.
- Baker et al. 2010. Implementing Routine Testing for Severe Combined Immunodeficiency within Wisconsin's Newborn Screening Program. Public Health Rep. 125 Suppl 2, 88-95.
- Verbsky et al. 2012. Newborn Screening for Severe Combined Immunodeficiency; The Wisconsin Experience (2008-2011). J. Clin. Immunol. 32, 82-88.

Trouble Shooting Guide

Problem	Possible Cause	Solution
No PCR product or non-specific product (or signal) from positive control samples using purified genomic DNA	PCR primers, reagents or cycling conditions were not optimal	Refer to the appropriate PCR reagent product manual to optimize PCR conditions
No PCR product or non-specific product (or signal) from extracts	Inadequate extract heating	Ensure that DBS punch extracts are incubated at ≥95°C. Program thermal cycler for 96°C.
	Extraction time was too short	Incubate sample in extraction reagent for up to 30 minutes at 95°C.
	Too much extract or template in PCR	Use less than 1/10 volume of extract in the PCR reaction. Extracts can be diluted 5-, 10-, 20-fold or more in TE buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) prior to PCR.