Extracta DNA Prep for PCR - Tissue

Cat. No	95091-002	Size:	2.5 mL	**Store at room temperature**
	95091-025		25 mL	
	95091-250		250 mL	

Description

The Extracta DNA Prep for PCR - Tissue consists of a two-component reagent kit for rapid and efficient extraction of PCR ready genomic DNA from mammalian tissues. The kit has been demonstrated to work with mouse tail biopsies, ear punches and a variety of human tissue samples including hair, buccal cells and saliva. Extracts are compatible with both endpoint PCR and real-time qPCR reagents including:

- AccuStart™ PCR SuperMix products for endpoint PCR
- AccuMelt™ HRM SuperMix products for high resolution melting analysis
- PerfeCTa® FastMix™ or PerfeCTa SuperMix products for SYBR® Green detection
- PerfeCTa qPCR FastMix or PerfeCTa qPCR SuperMix products for TaqMan® assays

Components

	95091-002	**95091-025**	**95091-250**
Extraction Reagent	1 x 2.5 mL	1 x 25 mL	2 x 125 mL
Stabilization Buffer	1 x 2.5 mL	1 x 25 mL	2 x 125 mL

Storage and Stability

Store components at room temperature.
For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form

Protocol

1. Add tissue samples to an appropriate volume of Extraction Reagent (refer to the guide below for recommended tissue sample sizes and Extraction Reagent volumes). Ensure that the tissue samples are small and completely submerged in Extraction Reagent.
2. Heat samples to 95°C for 30 minutes. **For a faster protocol see the Protocol Notes**.
3. Cool samples to room temperature and add an equal volume of Stabilization Buffer.
4. Use up to 5 µL of extract in a 50 µL PCR reaction. When scaling PCR reactions, add up to 1/10 volume of undiluted extract to the PCR reaction.

Tissue Sample Sizes and Extraction Reagent Volumes

Sample	Size	Volume	Comments
Mouse tails	0.2 to 0.5 cm	75 µL	Fresh or frozen tail samples can be used. Tail samples should be less than 0.5 cm.
Mouse ear punches	1 to 2 mm	50 µL	Ensure that the ear punches are completely submerged in Extraction Reagent.
Animal tissues	2 to 10 mg	100 µL	Tissue samples should be small and completely submerged in Extraction Reagent.
Buccal swabs	1 buccal brush or swab	250 µL	Collect cheek cells using a buccal brush or swab and place into Extraction Reagent in a 1.5 mL microcentrifuge tube. Twirl the brush or swab in the Extraction Reagent and carefully press and rotate the brush or swab against the side of the tube while removing it from the solution to ensure that most of the liquid remains in the tube.
Hair	1-3 hairs with root	75 µL	Trim hair shaft to 0.5 cm leaving the root intact and place root end down into Extraction Reagent.
Saliva	10-20 µL	100 µL	Mix samples well in Extraction Reagent.

Protocol Notes

- **Faster protocol:** Extraction incubation time can be shortened to 10 minutes depending on the tissue sample.
- **Maximum yield:** Tissue can be diced or smashed into smaller pieces to expose more surface area to the Extraction Reagent resulting in shorter extraction time and/or greater yield of extracted DNA. Extraction incubation time can be extended to 60 minutes. The yield of extracted DNA will generally increase with increased incubation time. Optimal extraction incubation time will depend on the tissue sample.

- **Simpler protocol for high throughput applications:** Tissue extracts in Extraction Reagent can be used directly in PCR reactions without the addition of Stabilization Buffer. Add up to 1/10 volume of the extract in PCR reactions immediately following extract preparation. Addition of Stabilization Buffer is required for long term storage of extracts.
- Tissue extractions can be done in tubes or multiwell plates and incubated in PCR machines.
- Extracts can be stored at 4°C or frozen at -20°C for several months or longer provided Stabilization Buffer has been added. It is not necessary to remove residual tissue from extracts.
- Extracts can be diluted 5-, 10-, 20-fold or more in H_20 or TE (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) prior to PCR.

Reagents and Equipment Required but not Provided

- Microcentrifuge tubes (0.5 mL or 1.5 mL), PCR tubes (0.2 mL) or multiwell plates
- PCR reagents
- Heat block and/or thermal cycler

Precautions and Disclaimer

This product is for research use only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

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 tissue extracts	Too much tissue in extraction	Use less tissue or cut tissue into smaller pieces. Ensure that the entire tissue sample is submerged in Extraction Reagent
	Inadequate extract heating	Ensure that tissue extracts are incubated at 95°C.
	Extraction time was too short	Incubate tissue in Extraction Reagent for up to 60 minutes at 95°C.
	Too much extract 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 H_20 or TE (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) prior to PCR.

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