AccuStart™ II Mouse Genotyping Kit

Cat. No. 95135-100 Size: 100 reactions
95135-500 500 reactions

Store at -25ºC to -15°C
(see Storage and Stability notes)

Description

The AccuStart II Mouse Genotyping Kit is designed for fast and easy preparation of PCR-ready DNA extracts and endpoint PCR analysis from tissues such as tail snips and ear punches commonly used for genotyping knockout and transgenic animals. The kit combines Extracta™ DNA Prep for PCR - Tissue with AccuStart II GelTrack® PCR SuperMix in a single kit. Extracta reagents allow rapid extraction of PCR ready DNA that can be used directly in PCR reactions eliminating time consuming or expensive purification steps. AccuStart II GelTrack PCR SuperMix has been optimized for genotyping applications that commonly utilize 3 or more primers in multiplex PCR reactions that allow amplification and analysis of two or more targets in a single reaction such as normal and mutant alleles of a gene knockout or that determine the presence or absence of transgene constructs in transgenic animals. AccuStart II GelTrack PCR SuperMix contains all components for PCR (except primers and template) simplifying reaction assembly, improving assay reproducibility, reliability and reducing the risk of contamination. The PCR SuperMix is a 2X concentrated, ready-to-use reaction cocktail for routine endpoint PCR amplification of DNA fragments up to 4kb and subsequent analysis by agarose gel electrophoresis. The PCR SuperMix additionally includes electrophoresis-tracking dyes that migrate at approximately 4 kb and 50 bp to allow direct loading of PCR product on agarose gels following amplification. AccuStart Taq DNA polymerase in the master mix is inactivated with monoclonal antibodies that bind the polymerase and keep it inactive prior to the initial PCR denaturation step. Upon heat activation (1 minute at 94ºC), the antibodies denature irreversibly, releasing fully active, unmodified Taq DNA polymerase. This enables specific and efficient primer extension with the convenience of room temperature reaction assembly.

Components

	95135-100	**95135-500**	
Extraction Reagent	8 x 1.25mL	2 x 25mL	To reorder separately, use Cat. No. 95091
Stabilization Buffer	8 x 1.25mL	2 x 25mL	
AccuStart II GelTrack PCR SuperMix	1 x 1.25mL	5 x 1.25mL	To reorder separately, use Cat. No. 95136

Storage and Stability

Remove AccuStart II GelTrack PCR SuperMix from the kit box and store separately.
Store AccuStart II GelTrack PCR SuperMix in a constant temperature freezer at -25°C to -15°C upon receipt. Repeated freezing and thawing does not impair product performance.
Extracta Reagents can be stored at room temperature. If frozen, mix thoroughly after thawing before use.
For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form

DNA Extraction

1. Add tissue samples to an appropriate volume of Extraction Reagent (see table below). Ensure that tissue samples are **small and completely submerged** in Extraction Reagent.
2. Heat samples to 95°C for 30 minutes (tissue samples will not dissolve and appear to remain intact after incubation – this is normal).
3. Cool samples to room temperature and add an equal volume of Stabilization Buffer (safe stopping point). Extracts can be stored at 4°C for several weeks or at -20°C for several months.
4. Use up to 2.5 µL of extract in a 25 µL PCR reaction (see PCR Reaction Setup below). Depending on the sample size and extraction conditions the extract may require dilution in water or TE buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) for optimal PCR results.
5. Load 5 or 10 µL (or as appropriate) on an agarose gel.

Extraction Protocol Notes

The protocol is flexible and can be optimized for individual applications, for example, by shortening the incubation time at 95°C and/or addition of extract directly to the PCR without addition of Stabilization Buffer. The use of PCR tubes or multi-well plates and incubation in a thermal cycler with a heated lid is recommended to increase throughput and to prevent the sample from condensing on the lid.

Tissue Sample Sizes and Extraction Reagent Volumes

Sample	Size	Volume	Comments
Tail snips	2 mm	100 µL	Fresh or frozen tail snips can be used. Use proportionally more Extraction Reagent with tail snips larger than 2 mm. Samples will appear to remain intact and will not dissolve in Extraction Reagent after heating.
Ear punches	2 mm	50 µL	Ensure that the ear punches are completely submerged in Extraction Reagent.
Other tissues	5 mg	100 µL	Tissue samples should be **small** and completely submerged in Extraction Reagent.

PCR Reaction Setup

Component	Volume for 25-µL rxn.	Final Concentration
AccuStart II GelTrack PCR SuperMix (2X)	12.5 µL	1x
Forward primer	variable	100 – 500 nM
Reverse primer	variable	100 – 500 nM
Nuclease-free water	variable	
Extracta DNA Extract	up to 2.5 µL	Variable
Final Volume (µL)	25 µL	

PCR Cycling Conditions

Incubate the completed reaction mix in thermal cycler as follows:

Initial denaturation:	94ºC, 1 to 3 min
PCR cycling (30-40 cycles:)	94ºC, 10 to 30 s 55 – 65ºC, 15 to 30s 68 – 72ºC, 1 min per kb of product length
Hold	4ºC - 10ºC until processed for agarose gel analysis

Full activation of Taq DNA polymerase occurs within 30 seconds at 94ºC. Complete denaturation of dsDNA target is important for efficient PCR amplification and may require different initial denaturation times depending on the properties of a given target sequence.

PCR Protocol Notes

For larger or smaller reaction volumes scale all components proportionally. Preparation of a reaction cocktail is recommended to reduce pipetting errors and maximize assay precision. Assemble the reaction cocktail with all required components except DNA extract and dispense equal aliquots into each reaction tube or well. Add extract as the final step. Addition of samples in 5 to 10 µL volumes will improve assay precision.

Quality Control

Kit components are free of contaminating DNase and RNase. AccuStart II GelTrack PCR SuperMix is functionally tested for amplification of a 4 kb fragment from a single-copy gene in human genomic DNA.

Trouble Shooting Guide

Problem	Possible Cause	Solution
No PCR product or non-specific products are visible on the gel	PCR primers or cycling conditions were not optimal	Re-design PCR primers or change PCR cycling conditions
	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 30 minutes at 95°C.
	Too much extract in PCR	Dilute extracts 1/5, 1/10 or 1/20 in water or TE buffer (10 mM Tris- HCl pH 8.0, 0.1 mM EDTA) prior to PCR.
PCR product produces a smear in the gel	Too much extract in PCR	Dilute extracts in water or TE buffer prior to PCR

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