

Check the product label for actual catalog number, lot and expiry date.

## SampleIN™ Direct PCR Kit

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
DPK0101	80 r of 50 µl	1.6 ml - DPK Lysis Buffer, 5X 0.8 ml - DPK Protease Buffer, 10X 2 x 1 ml - ALLin™ HS Red Taq Mastermix, 2X	DPK Lysis Buffer, 5X contains all components required for an efficient lysis of mammalian tissue samples. DPK Protease Buffer, 10X contains proteases to eliminate sample proteins.
DPK0105	400 r of 50 µl	5 x 1.6 ml - DPK Lysis Buffer, 5X 5 x 0.8 ml - DPK Protease Buffer, 10X 10 x 1 ml - ALLin™ HS Red Taq Mastermix, 2X	1X ALLin™ HS Red Taq Mastermix contains hot-start enzyme, 0.25 mM dNTPs, 3 mM MgCl <sub>2</sub> , enhancers, stabilizers, red electrophoresis tracking dye and density reagents for gel loading

Storage In the dark at -20°C.

### APPLICATIONS

- Fast direct PCR without template purification
- Mouse genotyping and knockout analysis
- Direct PCR from mouse tail or ear, mammalian tissues (including FFPE), hair follicle, buccal swabs and blood (including EDTA or FTA samples), plants

### PRODUCT DETAILS

SampleIN™ Direct PCR Kit is a premium tool for a fast direct PCR eliminating the need of tedious template purification. The kit is excellent for direct PCR from mouse tail or ear, mammalian tissues, hair follicle, buccal swabs and blood.

Rapid 15 min DNA extraction using DPK Lysis and Protease Buffers in a single tube generates PCR template extract which is further amplified under fast cycling conditions with a hot-start Taq master mix that includes red dye for direct gel loading.

In a 2% agarose TAE gel the red dye migrates with ~350 bp DNA, in 1% agarose TAE gel with ~ 600 bp DNA fragments.

The ALLin™ HS Red Taq Mastermix includes a hot start Taq DNA Polymerase what ensures high yield, specific, low background amplification. Mix components allow for a fast PCR cycling and increase success when working with complex templates or multiplexing with up to 3 primer pairs.

Generated A-tailed PCR products are suitable for ligating into TA cloning vectors, sequencing and other applications.

### BENEFITS

- Ready-to load PCR in 50 minutes without template purification
- Single-tube 15 min DNA extraction combined with fast hot-start PCR
- Red dye in the PCR master mix for direct gel loading
- High yields under standard or fast cycling conditions
- Success with GC/AT rich templates

### SAMPLE GUIDELINES

Sample (fresh or frozen)	Amount	Extraction vol.
Mouse tail	2 mm or 3-5 mg	100 µl
Mouse ear	2 mm <sup>2</sup> or 3-5 mg	100 µl
Mammalian tissue	5 mg	100 µl
FFPE Tissue	2 mm <sup>2</sup> of 10 µm section	100 µl
Blood (fresh/EDTA)	2 µl	100 µl
Blood Guthrie cards	2 mm <sup>2</sup>	100 µl
Blood FTA/FTA Elute cards	2 mm <sup>2</sup>	100 µl
Hair follicle	2 follicles	100 µl
Buccal swab	1 swab	300 µl

Sample amounts can be slightly increased for better yields, but too much material may cause inefficient lysis and PCR inhibition.



SampleIN™ Direct PCR Kit (upper) gives higher PCR yields from different dilutions of mouse tail sample extracts compared to competitors' kits (lower).

### I. SAMPLE DNA EXTRACTION PROTOCOL

- Take typical measures to prevent contamination, keep your bench clean, wear gloves, and use sterile tubes.
- Thaw DPK Buffers at room temperature. Mix well before use.
- Prepare a 100 µl extraction reaction in a sterile vial (use 3x larger volumes of all reagents for buccal swab):

Sample	As indicated above in SAMPLE GUIDELINES
DPK Lysis Buffer, 5X	20 µl
DPK Protease Buffer, 10X	10 µl
PCR Water (not supplied)	70 µl

- Mix very gently. Place into the thermal block/water bath set like:
- |                       |  |
|-----------------------|--|
| Lysis                 | 75°C - 5 min. Vortex twice during lysis. |
| Protease inactivation | 95°C - 10 min                            |
- Add 900 µl of PCR Water. Centrifuge 1 min to pellet cell debris.
  - Remove supernatant into the sterile tube.
  - Store it at -20°C for several months or use immediately for PCR.

IN VITRO RESEARCH USE ONLY

### II. PCR PROTOCOL

- Include a no-template control and positive control in parallel.
- Thaw and keep PCR reagents on ice. Mix well before use.

✓ Prepare a 50 µl PCR reaction:

Rev. & For. Primers	0.1-0.4 µM final each (≤ 2 µl of 10 µM)
Template	1-5 µl of extraction supernatant
PCR Water	to 25 µl
ALLin™ HS Red Taq Mastermix, 2X	25 µl

✓ Mix gently. Place into the PCR instrument set like:

Initial denaturation	1 cycle: 95°C - 2 min
Denaturation	40 cycles: 95°C - 15 sec
Annealing	40 cycles: 55-65°C - 15 sec
Extension	40 cycles: 72°C - 15 sec/kb (90 sec for multiplex)

Load probes on the gel. The red loading dye is included in PCR mix. In a 2% agarose TAE gel the red dye migrates with ~350 bp DNA, in 1% agarose TAE gel with ~ 600 bp DNA fragments.

Store probes for short time on ice, for long at -20°C.