# 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	DPK Lysis Buffer, 5X contains all components required for an efficient lysis of mammalian tissue samples.
		2 x 1 ml - ALLin™ HS Red Taq Mastermix, 2X	DPK Protease Buffer, 10X contains proteases to eliminate sample proteins.
	400 r of 50 μl	5 x 1.6 ml - DPK Lysis Buffer, 5X	1X ALLin™ HS Red Taq Mastermix contains hot-start enzyme, 0.25 mM
DPK0105		5 x 0.8 ml - DPK Protease Buffer, 10X	dNTPs, 3 mM MgCl $_{2}$ , enhancers, stabilizers, red electrophoresis tracking dye
		10 x 1 ml - ALLin™ HS Red Taq Mastermix, 2X	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 Tag 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 μΙ
Mouse ear	2 mm <sup>2</sup> or 3-5 mg	100 μΙ
Mammalian tissue	5 mg	100 μΙ
FFPE Tissue	2 mm <sup>2</sup> of 10 µm section	100 μΙ
Blood (fresh/EDTA)	2 µl	100 μΙ
Blood Guthrie cards	2 mm <sup>2</sup>	100 μΙ
Blood FTA/FTA Elute cards	2 mm <sup>2</sup>	100 μΙ
Hair follicle	2 follicles	100 μΙ
Buccal swab	1 swab	300 μΙ

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).

### 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):

	Cample	As indicated above in
	Sample	SAMPLE GUDELINES
	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

- $\bullet\,$  Add 900  $\mu I$  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

### 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	25 μl
Mastermix, 2X	
✓ 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.

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