TECHNICAL MANUAL

horizon™
INSPIRED CELL SOLUTIONS

E. coli Keio Knockout Collection

Product description

The *E. coli* Keio Knockout Collection is a set of precisely defined, single-gene deletions of all nonessential genes in *Escherichia coli* K-12. Of 4288 genes targeted, mutants were obtained for 3985 genes. Two independent mutants were obtained for each deleted gene, yielding a total of 7970 knockout strains (Baba *et al.* 2006).

Using λ Red recombination, a FRT-flanked kanamycin cassette was used to replace each coding region. This cassette may be excised by FLP recombination, leaving an in-frame, translatable sequence that includes the endogenous start, a short recombinational scar sequence, and an 18-nucleotide, C-terminal coding sequence from the endogenous gene. For details concerning cassette excision, please consult Datsenko and Wanner (2000).

This collection is a new resource for systematic analyses of unknown gene functions and gene regulatory networks, but also for genome-wide testing of mutational effects in a common strain background, E. coli K-12 BW25113.

Strain information

Knockouts are in the *E. coli* K-12 background strain BW25113. Genotype of BW25113: *rrnB3 ΔlacZ4787 hsdR514 Δ(araBAD)567 Δ(rhaBAD)568 rph-1*

Antibiotic resistance

E. coli K-12 knockout strains contain 1 antibiotic resistance marker (Table 1).

Replication protocol

For archive replication, grow all E. coli Keio knockout clones at 37 °C in 2x LB broth (low salt)* medium plus 25 µg/mL kanamycin. Prepare medium with 8% glycerol and the appropriate antibiotics.

1L LB broth (low salt) medium preparation

LB-Broth (low salt) medium	20 g/L
dH_2O	920 mL

Appropriate antibiotic(s) at recommended concentration(s).

Table 1. Antibiotic resistances.

Antibiotic	Concentration	Utility)
Kanamycin	25 µg/mL	Bacterial selection marker

Replication of plates

Prepare target plates by dispensing ~160 µl of 2x LB broth (low salt)* medium supplemented with 8% glycerol and appropriate antibiotic (25 µg/mL kanamycin).

***1x LB medium can be used instead of 2x LB broth medium.**

Prepare source plates

1. Remove foil seals while the source plates are still frozen. This minimizes cross-contamination.
2. Thaw the source plates with the lid on. Wipe any condensation underneath the lid with a paper wipe soaked in ethanol.

Replicate

1. Gently place a disposable replicator in the thawed source plate and lightly move the replicator around inside the well to mix the culture. Make sure to scrape the bottom of the well.
2. Gently remove the replicator from the source plate and gently place in the target plate and mix in the same manner to transfer cells.
3. Dispose of the replicator.
4. Place the lids back on the source plates and target plates.
5. Repeat steps 1–4 until all plates have been replicated.
6. Return the source plates to the –80 °C freezer.
7. Place the inoculated target plates in a 37 °C incubator without shaking for 18–19 hours.

Freeze at –80 °C for long term storage. Avoid long periods of storage at room temperature or higher in order to control background recombination products.

What clones are part of my collection?

A USB drive containing the data for this collection will be shipped with each collection. This file contains the location and accession number for each construct in the collection. The data file is also available at here.

Which antibiotic should i use?

You should grow all *E. coli* Keio knockout clones at 37 °C in LB broth (low salt) medium plus 25 µg/mL kanamycin.

How can i get rid of the kan cassette?

For excision of the cassette, you will need to transform with a FLP expression plasmid. Please see Datsenko and Wanner (2000) for details on how to perform the excision.

Table 2. Related products.

Product	Cat #
E. coli K-12 BW25113 Parental Strain	OEC5042

References

1. Baba T, Ara T, *et al.* (2006). Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol. Syst. Biol* **2**: 2006.0008.
2. Datsenko KA and BL Wanner (2000). One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *PNAS* **97**: 6640–6645.

If you have any questions, contact

t +44 (0) 1223 976 000 (UK) **or** +1 800 235 9880 (USA); +1 303 604 9499 (USA)
f + 44 (0)1223 655 581
w horizondiscovery.com/contact-us **or** dharmacon.horizondiscovery.com/service-and-support
Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

All trademarks are the property of Horizon Discovery Company unless otherwise specified. ©2018 Horizon Discovery Group Company—All rights reserved. First published February 2015. UK Registered Head Office: Building 8100, Cambridge Research Park, Cambridge, CB25 9TL, United Kingdom.

horizon™ INSPIRED CELL SOLUTIONS

V20418