

## Homologous Recombination Assay Kit Product # 35600

## Product Insert

### INTENDED USE

Norgen's Homologous Recombination Assay Kit provides a rapid and sensitive tool for measuring the efficiency of homologous recombination in both bacterial and mammalian cells. The kit consists of 5 components: (1) dl-1 plasmid, (2) dl-2 plasmid, (3) positive control plasmid, (4) assay primers mixture, and (5) universal primers mixture. The assay is based on the co-transformation of the two dl plasmids into bacterial cells followed by the isolation of plasmid DNA and quantification of the homologous recombination efficiency through qPCR. In addition, PCR can be used for qualitative analysis of the recombination efficiency. The primer mixture provided with the kit is designed to amplify only the homologous recombination product but not the dl plasmids (dl-1 or dl-2). The two plasmids can be co-transfected into mammalian cells, then the DNA can be isolated and the homologous recombination efficiency can be assayed by qPCR (or PCR). In addition, the efficiency of homologous recombination can be assessed in bacteria by blue/white screening using X-gal. The provided positive control contains the functional LacZ sequence and can be used as a positive control for the plasmid DNA isolation efficiency (after transformation or transfection) or PCRs. The use of a PCR-based homologous recombination assay enhances sensitivity and eliminates the need for labor intensive screening by using traditional protein expression and staining techniques and is suitable for high throughput screening studies.

**The kit is designed to perform 200 transformations or 24 transfections.**

### Kit Components:

Component	Contents
dl-1 Plasmid	12 µg (50 ng/µL)
dl-2 Plasmid	12 µg (50 ng/µL)
Positive Control Plasmid	5 µg (50 ng/µL)
Assay Primers Mixture	400 µL
Universal Primers Mixture	400 µL
Nuclease-Free Water	1.25 mL
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### MATERIALS REQUIRED BUT NOT SUPPLIED

- Transformation or transfection reagents
- Plasmid isolation Kit or gDNA isolation kit
- PCR/qPCR reagents: we highly recommend using the following PCR master mixes:
  - o PCR: 2X PCR Master Mix (Norgen Biotek Cat# 28007) or Taq 2X Master Mix (NEB Cat# M0270L).
  - o qPCR: 2X Real-Time PCR Master Mix (Norgen Biotek Cat# 28322) or Luna® Universal qPCR Master Mix (NEB Cat# M3003S).
- PCR/qPCR Thermocycler

### SHELF LIFE AND HANDLING

All solutions should be kept tightly sealed and stored at -20°C or lower temperature. This kit is stable for 5 years after the date of shipment.

### QUALITY CONTROL

In accordance with Norgen's Quality Management System, each of Norgen's Homologous Recombination Assay Kit is designed for general laboratory purposes. The kit components undergo quality assurance testing to verify that its composition does not vary from lot to lot.

## ASSAY PROTOCOL:

- 1) Prepare your competent cells or mammalian cells and the suitable transformation or transfection reagents.
- 2) A- Transformation:  
For each bacterial transformation, use 25 ng (0.5  $\mu$ L) of each **dl plasmid (dl-1 and dl-2)** and mix well before transforming the entire mix. For the positive control sample, use 25 ng (0.5  $\mu$ L) of the **Positive Control Plasmid**. For negative control, use 25 ng (0.5  $\mu$ L) of each of the **dl plasmids** in separate transformations. Use 0.1 mg/mL of ampicillin for selection of transformed cells.  
  
B- Transfection:  
For each transfection (24 well plate well size), use 0.5  $\mu$ g (10  $\mu$ L) of each **dl plasmid (dl-1 and dl-2)** and mix well before transfecting the entire mix. For the positive control sample, use 0.5  $\mu$ g (10  $\mu$ L) of the **Positive Control Plasmid**. For negative control, use 0.5  $\mu$ g (10  $\mu$ L) of each of the **dl plasmids** in separate transfections.
- 3) After the desired time (minimum of 12 hours) and conditions, isolate the plasmid DNA using an appropriate isolation method (not provided).
- 4) Use 2  $\mu$ L of the provided **Assay Primers Mixture** for each 20  $\mu$ L PCR reaction **using the appropriate PCR/qPCR reagents** (not provided). For a PCR control 0.5 ng of the **Positive Control Plasmid** can be used.
- 5) PCR cycling temperature and time:  
A- qPCR: Initial denaturation at 95°C for 3 minutes then 95°C for 15 sec, 61°C for 15 sec, and 72°C for 15 sec, and repeated for 40 cycles then run the melt curve analysis.  
  
B- PCR: Initial denaturation at 95°C for 3 minutes then 95°C for 15 sec, 61°C for 15 sec, and 72°C for 15 sec, and repeated for 35 cycles then a final extension at 72°C for 5 min.
- 6) To detect the backbone plasmids (dl-1, dl-2 or the positive control), 2  $\mu$ L of the supplied **Universal Primers Mixture** can be used for each 20  $\mu$ L PCR reaction by following the same PCR settings in **Step 5**.
- 7) The expected amplicon sizes from the different plasmids are:  
A- Using the assay primer set:
  - i. - positive control: 420bp  
B- Using the universal primer set:
  - i. - dl-1 plasmid: 546bp
  - ii. - dl-2 plasmid: 183bp
  - iii. - positive control: 563bp

### Note:

It is important to keep a number of points in mind when performing PCR:

1. Using high quality, purified DNA templates greatly enhances the success of PCR.
2. Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination.
3. There should be designated solutions, nuclease-free water, tips, tubes, pipettes, etc. for PCR only.

Related Products	Product #
RNA/DNA/Protein Purification Kit	23500
Plasmid MiniPrep DNA Kit	13300
2X Real-Time PCR Master Mix	28322
2X PCR Master Mix	28007

### Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use. We reserve the right to change, alter, or modify any product to enhance its performance and design.

### Technical Assistance

NORGEN's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of NORGEN products. If you have any questions or experience any difficulties regarding Norgen's Homologous Recombination Assay Kit or NORGEN products in general, please do not hesitate to contact us.

NORGEN customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at NORGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362 or call one of the NORGEN local distributors ([www.norgenbiotek.com](http://www.norgenbiotek.com)) or through email at [techsupport@norgenbiotek.com](mailto:techsupport@norgenbiotek.com).

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