

# PathwayScan™ Single Pathway Reporter Kit



## **INSTRUCTION MANUAL**

**Catalog # GM8101~GM8146**

Revision A

For Research Use Only. Not for Use in Diagnostic Procedures



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# PathwayScan™ Single-Pathway Reporter Kit

## KIT CONTENTS

	<b>Catalog # GM8101 ~ GM8146 <sup>c</sup></b>
1 transcription factor reporter plasmid <sup>a,b</sup> 1 negative control reporter plasmid <sup>a,b</sup> 1 positive control reporter plasmid <sup>a,b</sup>	100ng/ul x 500ul for each of 3 reporter plasmids

<sup>a</sup> Each of the 3 plasmid contains a small amount of Renilla luciferase constitutive expression plasmid as internal transfection control.

<sup>b</sup> All plasmids are not supposed to be transformed and replicated in E.coli.

<sup>c</sup> This protocol is not included with product shipments. Please go to [www.gmbiosciences.com](http://www.gmbiosciences.com) to download it.

## STORAGE CONDITIONS

-20°C

## MATERIALS NEEDED BUT NOT PROVIDED

- **Cell line**
- **Cell culture reagents** such as medium, Fetal calf serum (FCS), antibiotics
- **96-well cell culture plate**
- **Multi-channel pipettor**
- **Transfection reagent** such as Lipofectamine 2000
- **Reporter assay reagents for Firefly luciferase and Renilla luciferase**
- **Luminometer**

# PathwayScan™ Reporters Introduction

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Transcription factor (TF) reporter plasmid expresses Firefly luciferase under control of the TF's binding sequences. The negative control plasmid is Firefly luciferase gene without any promoter, and the positive control plasmid expresses Firefly luciferase driven by CMV promoter.

Each of the 3 plasmids above is pre-mixed with Renilla plasmid which constitutively expresses Renilla luciferase. The expressed Renilla luciferase functions as an internal control to normalize variations of cells number and transfection efficiency among wells.

**Note** *All of the PathwayScan plasmids are not supposed for transformation and replication in E.coli.*

## PROTOCOL

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- 1. Pre-plate cells in 96-well cell culture plates 1 day prior to transfection, grow cultures to 60%~80% confluence.**

Cell lines that are easily transfected are preferred. For example, adherent 293H, 293F, CHO-S, Cos-7L and HeLa cells can be easily transfected by Lipofectamine 2000.

Pre-plating is not required to transfect suspension cells and some adherent. For example, adherent cell line 293H, 293F, CHO-S and Cos-7L work very well without pre-plating for Lipofectamine 2000 transfection. It should be determined empirically whether pre-plating is needed for specific cell lines and transfection reagents.

It is recommended to culture the cells in medium supplemented with 10% FCS during and after transfection if the cells are normally cultured in the presence of serum. High basal signals can be induced by FCS, which is good for detecting inhibitory effect. However, if only activation effects are desired, 0.5% FCS can be used after transfection, which may give lower basal signals.

It should be cautious in using antibiotics during transfection. However, some cell lines such as HEK293 cells tolerate antibiotics very well. Whether to use antibiotics during transfection should be determined empirically according to cell lines and transfection reagents.

- 2. Prepare transfection complexes of 3 reporter plasmids respectively.**

Perform transfection when cells become 60%-80% confluence. Choose desired transfection reagent. Lipofectamine 2000 has been tested and is recommended.

The amount of DNA to use in transfection should be determined according to manufacture manual of transfection reagent. For example, if Lipofectamine 2000 is used to transfect HEK 293 cells, 0.1ug reporter plasmid is needed for each well; 0.6ug of each 3 reporter plasmids is needed for an experiment in triplicate with two conditions.

A small amount of GFP (0.001~0.005ug) may be mixed with each of 3 plasmids to visually confirm transfection using fluorescent microscope. However, it should be cautious to co-transfect GFP because GFP is reported to have potential toxic to cells in some cases and it can induce oxidative stress.

If co-transfection is required, the amount of an interested plasmid should be same as that of PathwayScan reporter plasmid (1:1 ratio). For example, if Lipofectamine 2000 is used to transfect HEK 293 cells, 0.1ug interested plasmid and 0.1ug reporter plasmid should be co-transfected in each well.

Co-transfection of miRNA/siRNA/shRNA with PathwayScan reporter plasmid is feasible. Please refer to manufacture protocol of transfection reagent.

Co-transfection of peptide/protein with PathwayScan reporter plasmid is feasible. Please refer to manufacture protocol of transfection reagent.

### **3. Transfection by adding transfection complexes into cell culture respectively.**

For a two-condition experiment in triplicate, each of transfection complexes prepared above should be added into 6 wells in equal amount.

Generally, it is not necessary to remove complex or change/add medium after transfection. Please see manufacture protocol of transfection reagent for details. If Lipofectamine 2000 is used, changing/adding medium post transfection is not needed, but transfection complex may be removed after 4-6 hours.

It is not recommended to change medium for cell line, such as HEK 293, which is loosely bound to culture plate.

### **4. Continue culturing cells for 16 ~ 24 hours, then stimulate the cells.**

If stimulation is required, directly adding stimulant into culture medium at 16 hours post transfection and continue cultures for at least 5 hours before harvesting.

Changing medium is generally not necessary for stimulation. However, whether to change medium for stimulation should be determined empirically.

It is not recommended to change medium for cell line, such as HEK 293, which loosely bound to culture plate.

#### **5. Lyse cells, test Firefly luciferase activity and Renilla luciferase activity for each well.**

Remove culture medium to harvest cells. Add 100ul of lysis buffer (see PREPARATION OF SOLUTIONS for recipe) into each well. Slightly rock the plate several times from side to side, and stand at room temperature for 5~10min to lyse the cells.

In order to increase pipetting accuracy, it is recommended to remove genome DNA (sticky materials) out of lysate. Insert a tip into lysate and swirl the tip 5-10 times. A small mess of sticky transparent materials will stick to the end of the tip. Pull out the tip and discard it. The remaining lysate is ready for testing luciferase activity.

Luciferase reagents (including luciferin and co-elenterazine) can be either commercial or home-made. Luciferin is the substrate for firefly luciferase's bioluminescence reaction, while co-elenterazine is the substrate for renilla luciferase activity. Promega's Dual-Luciferase system (either E1910 or E2920) uses identical lysate (in a single vial) to test activity of Firefly luciferase and activity of Renilla luciferase sequentially, which reduces the pipetting variations. E1910 is compatible with luminometer equipped with auto-injector. E2920 generates luminescence in a very stable manner, and is compatible with any luminometer.

For using home-made luciferase reagents, prepare luciferin reaction solution and co-elenterazine reaction solution respectively according to traditional recipe (not provided). However, home-made reagents are much less sensitive than Promega's Dual-Luciferase system, and are thus not recommended.

#### **6. Data analysis**

Firefly luciferase activity represents TFs activity, whereas Renilla luciferase functions as internal control to normalize variations of cell-seeding and transfection among wells. It is recommended to normalize the activity of Firefly luciferase to that of Renilla luciferase.

## PREPARATION OF SOLUTIONS

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### Lysis buffer

10mM TrisHCl pH7.5

2mM DTT

1% TritonX100

2mM EDTA

## MSDS INFORMATION

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The Material Safety Data Sheet (MSDS) information is available on the web at <http://www.gmbiosciences.com>. MSDS documents are not included with product shipments.

## LIMITED PRODUCT WARRANTY

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## ORDERING INFORMATION AND TECHNICAL SUPPORT

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