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Data Sheet

The Transfection Collection™ – Myc Transient Pack
(Myc signaling pathway)
Catalog #: 79284

Background

The Myc signaling pathway plays an important role in cell proliferation, differentiation, transformation and apoptosis. The c-Myc protein is a transcription factor that heterodimerizes with Max to regulate Myc signaling pathway responsive genes. Myc mutations have been linked to the development of a number of human cancers, including Burkitt's lymphoma, cervical, ovarian, breast, lung and pancreatic carcinoma, making Myc a promising therapeutic target for cancer treatment.

Description

The Myc Transient Pack is designed to provide the tools necessary for transiently transfecting and monitoring the activity of the Myc signaling pathway in cultured HCT116 cells. The Myc Transient Pack contains transfection-ready vectors containing firefly luciferase as a Myc pathway-responsive reporter and constitutively expressing Renilla luciferase as a transfection control. It also includes the TWO-Step Luciferase detection reagents to detect both luciferase activities and specialized medium for growing and assaying HCT116 cells.
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The key to the Myc Transient Pack is the expression vectors for c-Myc and Myc luciferase reporter vector. Inside the cells, c-Myc will bind to Max, translocate to the nucleus, and induce expression of the Myc luciferase reporter vector. This reporter contains the firefly luciferase gene under the control of multimerized Myc responsive elements located upstream of a minimal promoter. The Myc reporter is premixed with constitutively-expressing *Renilla* (sea pansy) luciferase vector, which serves as an internal positive control for transfection efficiency.

The pack also includes a non-inducible firefly luciferase vector premixed with constitutively-expressing *Renilla* luciferase vector as a negative control. The non-inducible luciferase vector contains a firefly luciferase gene under the control of a minimal promoter, but without any additional response elements. This negative control is critical for determining pathway-specific effects and background luciferase activity. An "empty" expression vector without the c-Myc gene is also provided as an additional negative control.

Additionally, the pack includes cell culture medium that has been optimized for use with HCT116 cells*. HCT116 is a human colon cancer cell line with a mutated β -catenin which leads to the accumulation of β-catenin and constitutive activation of the Myc signaling pathway. This medium includes 10% fetal bovine serum, and 1% Pen/Strep. Finally, the pack provides

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the TWO-Step Luciferase (Firefly & Renilla) Assay System. These reagents provide highly sensitive, stable detection of firefly luciferase activity and Renilla luciferase activity. The TWO-step luciferase reagents can be used directly in cells in growth medium, and can be detected with any luminometer; automated injectors are not required.

*Note: the kit may be used with other cell lines than HCT116, but an alternate cell culture medium may be required for optimal cell growth. For HEK293 cells, we recommend using BPS Medium 1 (BPS Bioscience, #79259).

Applications

- Monitor Myc signaling pathway activity.
- Screen activators or inhibitors of the Myc signaling pathway.
- Study effects of RNAi or gene overexpression on the activity of the Myc pathway.

Components

Component	Amount	Storage
Reporter (Component A)(Component A)** Myc luciferase reporter vector + constitutively-expressing *Renilla* luciferase vector	500 µl (60 ng DNA/µl)	-20°C
Negative Control Reporter (Component B)(Component B)** Non-inducible luciferase vector + constitutively-expressing *Renilla* luciferase vector	500 µl (60 ng DNA/µl)	-20°C
c-Myc Expression Vector (Component C)(Component C)**	2 x 250 µl (100 ng DNA/µl)	-20°C
Negative Control Expression Vector (Component D)(Component D)** Empty expression vector without c-Myc	2 x 250 µl (100 ng DNA/µl)	-20°C
Firefly Luciferase Reagent Buffer	10 ml	-20°C
Firefly Luciferase Reagent Substrate (100x)	100 µl	-20°C *Protect from light*
Renilla Luciferase Reagent Buffer	10 ml	Room Temp.
Renilla Luciferase Reagent substrate (100x)	100 µl	-20°C *Protect from light*
BPS Medium 7	100 ml	+4°C

**These vectors are designed for use in transient transfections. They are NOT SUITABLE for transformation and amplification in bacteria.

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Materials Required but Not Supplied

- HCT116 cells. Other mammalian cell lines may also be used, but an alternate cell culture medium may be required for optimal cell growth; for HEK293 cells, we recommend BPS Medium 1 (BPS Bioscience, #60187).
- 96-well tissue culture plate or 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- Transfection reagents for mammalian cell line. We use Lipofectamine™ LTX with PLUS™ reagent (Life Technologies #15338100) or Lipofectamine™ 2000 (Life Technologies # 11668027). However, other transfection reagents should work equally well.
- Opti-MEM I Reduced Serum Medium (Life Technologies #31985-062)
- Luminometer

Generalized Transfection and Assay Protocols

The following procedure is designed to transfect the reporter into HCT116 cells using Lipofectamine LTX in a 96-well format. To transfect cells in different tissue culture formats, adjust the amounts of reagents and cell number in proportion to the relative surface area. If using a transfection reagent other than Lipofectamine LTX, follow the manufacturer's transfection protocol. Transfection conditions should be optimized according to the cell type and study requirements.
Note: This protocol is for HCT116 cells. For HEK293 cells, we use BPS Medium 1 (BPS Bioscience, #60187) as growth medium, and Lipofectamine™ 2000 for transfection, according to the manufacturer's protocol.

All amounts and volumes in the following setup are provided on a per well basis.

1. One day before transfection, seed cells at a density of ~ 30,000 cells per well in 100 µl of BPS Medium 7 so that cells will be 80-90% confluent at the time of transfection.

2. The next day, for each well, prepare complexes as follows:

   a. Dilute DNA mixtures in 20 µl of Opti-MEM I medium (antibiotic-free). Mix gently. Depending upon the experimental design, the DNA mixtures may be any of the following combinations:
      - 1 µl of **Reporter** (component A); in this experiment, the control transfection is 1 µl of **Negative Control Reporter** (component B).
      - 1 µl of **Reporter** (component A) + **c-Myc Expression Vector** (component C); in this experiment, the control transfections are: 1 µl of **Reporte**r (component A) + **Negative Control Expression Vector** (component D), 1 µl of **Negative Control Reporter** (component B) + **c-Myc expression vector** (component C), and 1 µl of **Negative**

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Control Reporter (component B) + **Negative Control Expression vector** (component D).

- 1 µl of **Reporter** (component A) + specific siRNA; in this experiment, the control transfections are: 1 µl of **Reporte**r (component A) + negative control siRNA, 1 µl of **Negative Control Reporter** (component B) + specific siRNA, and 1 µl of **Negative Control Reporter** (component B) + negative control siRNA.

Note: we recommend setting up each condition in at least triplicate, and preparing a transfection cocktail for multiple wells to minimize pipetting errors.

b. Add 0.1 µl of PLUS reagent to diluted DNA, mix gently. Incubate 10 minutes at room temperature.

c. After the 10 minute incubation, add 0.6 µl Lipofectamine LTX reagent to the diluted DNA. Mix gently and incubate for 25 minutes at room temperature.

3. Carefully remove and discard 20 µl of media from each of the wells of cell culture, taking care not to disturb the cells or touch the bottom of the well with the pipet tip. Add the 20 µl of complexes to each well containing 80 µl cells and medium. Mix gently by tapping the plate.

4. Incubate cells at 37°C in a 5% CO_2 incubator. After ~24 hours of transfection, change medium to fresh BPS Medium 7. ~48 hours after transfection, perform the TWO-Step Luciferase Assay System (below).

To study the effect of activators / inhibitors on the pathway, treat the cells with tested activator/inhibitor after 24 hours of transfection. Perform TWO-step luciferase assay ~48 hours after transfection (below).

TWO-Step Luciferase Assay Procedure

1. Thaw **Firefly Luciferase Reagent Buffer** by placing the reagent in a room temperature water bath. Equilibrate the buffer to room temperature and mix well before use. *Note: It is important that the **Firefly Luciferase Reagent Buffer** be at room temperature before use.*
2. Calculate the amount of Firefly Luciferase Assay Working solution needed for the experiment (**Firefly Luciferase Reagent Buffer** + **Firefly Luciferase Reagent Substrate**). Immediately prior to performing the experiment, prepare the Firefly Luciferase Assay Working Solution by diluting **Firefly Luciferase Reagent Substrate** into **Firefly Luciferase Reagent Buffer** at a 1:100 ratio and mix well. Avoid exposing to excessive light*. Only use enough of each component for the experiment, remaining* **Firefly Luciferase Reagent Buffer** *and* **Firefly Luciferase Reagent Substrate** *should be stored separately at* -20°C*.*
3. Remove multi-well plate containing mammalian cells from incubator. *Note: plates must be compatible with luminescence measurement with luminometer being used.*

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4. Add equal volume of Firefly Luciferase Assay Working Solution (step 2) to the culture medium in each well. Example: 96-well plate with 100 µl of culture medium requires 100 µl of Firefly Luciferase Assay Working Solution per well.

   Gently rock the plates for ≥15 minutes at room temperature. Measure firefly luminescence using a luminometer. The signal under these conditions will be stable for more than 2 hours at room temperature. For maximal light intensity, measure samples within 1 hour of reagent addition.

5. Calculate the amount of Renilla Luciferase Assay Working Solution needed for the experiment (**Renilla Luciferase Reagent Buffer** + **Renilla Luciferase Reagent Substrate**). Prepare the Renilla Luciferase Assay Working Solution by diluting **Renilla Luciferase Reagent Substrate** into **Renilla Luciferase Reagent Buffer** at a 1:100 ratio and mix well. Avoid exposing to excessive heat or light. *Only use enough of each component for the experiment,*

6. Add equal volume of Renilla Luciferase Assay Working Solution (step 5) to each well. Example: 96-well plate with 100 µl of culture medium + 100 µl Firefly Luciferase Reagent requires 100 µl of Renilla Luciferase Assay Working Solution per well.

7. Gently rock the plates for ~1 minute at room temperature. Measure Renilla luminescence using a luminometer.

8. Data analysis: subtract background (wells with medium and luciferase reagent only) from all the readings. To obtain the normalized luciferase activity for the Myc reporter, subtract the background luminescence, then calculate the ratio of firefly luminescence from Myc reporter to *Renilla* luminescence from the control *Renilla* luciferase vector. To determine the fold induction, divide the value for the treated cells by the value for the untreated cells.

Sample protocol to determine the effect of human c-Myc on Myc reporter activity in HCT116 and HEK293 cells

Additional materials required in this experiment setup:

- HCT116 cells. Alternately, HEK293 cells can be used along with BPS Medium 1 (BPS Bioscience, cat. #79259).
- Assay medium: Opti-MEM I (Life Technologies #31985-062) + 0.5% FBS + 1% Non-essential amino acids + 1 mM sodium pyruvate + 1% Pen/Strep

1. One day before transfection, seed HCT116 or HEK293 cells at a density of 30,000 cells per well into a white clear-bottom 96-well plate (Corning #3610) in 100 µl of BPS Medium 7 or 1, respectively. Incubate cells at 37°C in a CO_2 incubator overnight.

2. The next day, transfect 1 µl of **Reporter** (component A) or 1 µl **Negative Control Reporter** (component B) with 1 µl of **c-Myc Expression Vector** (component C) or **Negative Control Expression Vector** (component D) into cells following the procedure in **Generalized Transfection and Assay Protocols.**

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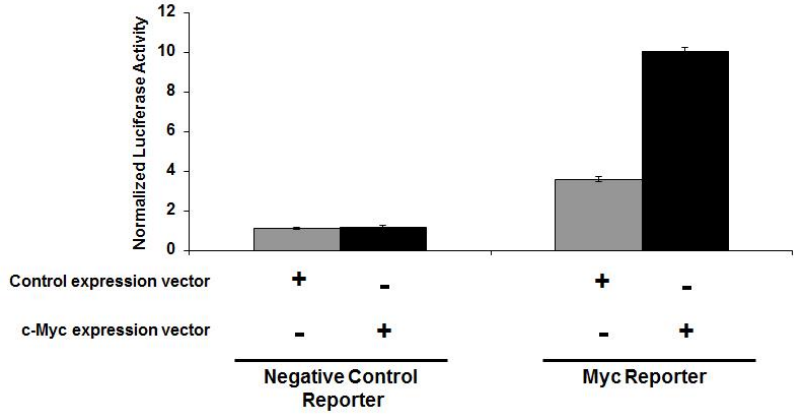
3. After ~24 hours of transfection, change medium in wells to 50 µl of assay medium. Add 50 µl of assay medium to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate. Incubate cells at 37°C in a CO_2 incubator for ~24 hours.
4. After ~48 hours of transfection, perform TWO-step luciferase assay as described above in TWO-Step Luciferase Assay Procedure. Briefly, dilute 100x Firefly Luciferase Reagent Substrate into Firefly Luciferase Reagent Buffer that has been equilibrated to room temperature. Add 55 µl of Firefly Luciferase reagent per well and rock at room temperature for ~15 minutes, then measure firefly luminescence using a luminometer. Dilute 100x Renilla Luciferase Reagent Substrate into Renilla Luciferase Reagent Buffer. Add 55 µl of Renilla Luciferase reagent per well, rock at room temperature for ~1 minute and measure Renilla luminescence.
5. To obtain the normalized luciferase activity for Myc reporter, subtract background luminescence, and then calculate the ratio of firefly luminescence from Myc reporter to *Renilla* luminescence from the control *Renilla* luciferase vector.

Figure 1. Activation of the Myc luciferase reporter by human c-Myc. The results are shown as normalized Myc luciferase reporter activity. Normalized luciferase activity is determined by dividing luciferase data with *Renilla* luciferase data. **(A)** HCT116 cells **(B)** HEK293 cells

A.



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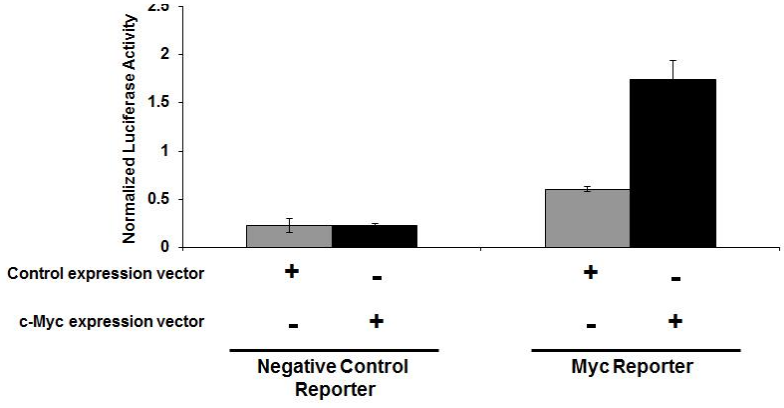

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Sample protocol to determine the effect of inhibitor ICG-001 on Myc reporter activity in HCT116 cells.

- Additional materials required in this experiment setupICG-001 (Selleck, #S2662): wnt-β-catenin pathway inhibitor. Myc can be activated by Wnt pathway.
- Assay medium: Opti-MEM I (Life Technologies #31985-062) + 0.5% FBS + 1% Non-essential amino acids + 1 mM sodium pyruvate + 1% Pen/Strep
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)

1. One day before transfection, seed HCT116 cells at a density of 30,000 cells per well into white clear-bottom 96-well plate in 100 µl of BPS Medium 7. Incubate cells at 37°C in a CO_2 incubator overnight.

2. Next day, transfect 1 µl of Myc luciferase reporter (component A) into cells following the procedure in **Generalized Transfection and Assay Protocols.**

3. After ~24 hours of transfection, remove medium in wells, add threefold serial dilution of ICG-001 in 50 µl of assay medium to inhibited wells; add 50 µl of assay medium to control wells; add 50 µl of assay medium to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate.

4. Incubate at 37°C in a CO_2 incubator for 18 hours.

5. Perform TWO-step luciferase assay as described above in **TWO-Step Luciferase Assay Procedure**. Briefly, dilute 100x Firefly Luciferase Reagent Substrate into Firefly Luciferase Reagent Buffer that has been equilibrated to room temperature. Add 55 µl of Firefly Luciferase reagent per well and rock at room temperature for ~15 minutes, then measure firefly luminescence using a luminometer. Dilute 100x Renilla Luciferase Reagent Substrate into Renilla Luciferase Reagent Buffer. Add 55 µl of Renilla Luciferase reagent per well, rock at room temperature for ~1 minute and measure Renilla luminescence.

6. To obtain the normalized luciferase activity for Myc reporter, subtract background luminescence then calculate the ratio of firefly luminescence from the Myc reporter to *Renilla* luminescence from the control *Renilla* luciferase vector. To determine the fold induction, divide the value for the treated cells by the value for the untreated cells.

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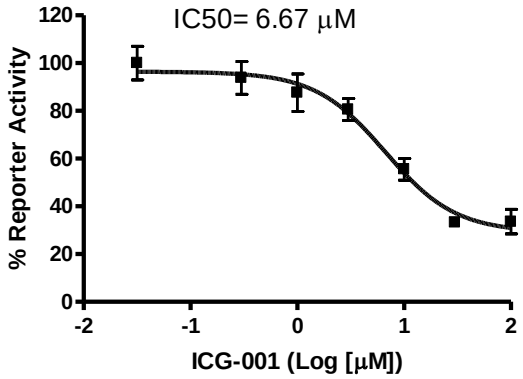
Figure 2. Dose response inhibition of constitutively active Myc reporter activity to inhibitor ICG-001 in HCT116 cells. The results are shown as percentage of Myc reporter activity. The normalized luciferase activity for Myc reporter transfected cells without ICG-001 treatment was set at 100%. The IC_{50} of ICG-001 is ~ 6.67 µM.



Reference

Pelengaris S, *et al*. (2002) c-MYC: more than just a matter of life and death. *Nat. Rev. Cancer*. **2(10):** 764-76.

Refills

Product Name	Catalog #	Size
Myc Reporter Kit	60519	500 rxns.
TWO-Step Luciferase (Firefly & Renilla) Assay System	60683-1	10 mL
TWO-Step Luciferase (Firefly & Renilla) Assay System	60683-2	100 mL
TWO-Step Luciferase (Firefly & Renilla) Assay System	60683-3	1 L
BPS Medium 7	79260	100 ml

Related Products

Product Name	Catalog #	Size
Myc Reporter (Luc) – HCT116 Cell Line	60520	2 vials
c-Myc, His-tag	40453	100 µg
BPS Medium 1	79259	100 ml