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AP-1 Luciferase Reporter-HEK293 Cell Line

Catalog number: RC1002

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

AP-1 Luciferase Reporter-HEK293 Cell Line

Catalog Number: RC1002, **Storage:** Immediately upon receipt, store in liquid nitrogen. (Ship on dry ice.)
Contents: Each vial contains 2 ~ 3 x 10^6 cells in 1 ml of 90% FBS + 10% DMSO.
Description: The AP-1 Luciferase Reporter cell line is a stably transfected HEK 293 cell line which expresses Renilla luciferase reporter gene under

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the transcriptional control of the activator protein 1 (AP-1). The AP-1 transcription factors are homo- or hetero-dimers that consist of proteins belonging to a group of structurally and functionally related members of the Jun family (c-Jun, JunB and JunD), the Fos (c-Fos, FosB, Fra-1 and Fra-2) and the subfamilies of ATF (ATFa, ATF-2 and ATF-3) and JDP (JDP-1 and JDP-2). The AP-1 induction by phorbol 12-myristate 13-acetate (PMA) is shown in Figure 1.

Applications: Functional Assay

Application Notes: Functional Assay, detecting the transcriptional activity of AP-1

Application Details:

Application:

Monitor the AP-1 signaling pathway activity.

Screen for activators or inhibitors of the AP-1 signaling pathway.

Culture conditions:

Cells should be grown at 37°C with 5% CO2 using DMEM medium supplemented with 10% FBS and 1% Pen/Strep, plus 3 µg/ml of Puromycin (Note: Puromycin can be omitted during the reporter cell assays).

It is recommended to quickly thaw the frozen cells upon receipt or from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of growth medium without Puromycin, spin down cells, resuspend cells in pre-warmed growth medium without Puromycin, transfer resuspended cells to T25 flask and culture in 37°C-CO2 incubator.

Leave the T25 flask in the incubator for 2~4 days without disturbing or changing the medium until cells completely recover viability and become adherent. Once cells are over 90% adherent, remove growth medium and passage the cells through trypsinization and centrifugation. At first passage, switch to growth medium containing Puromycin. Cells should be split before they reach complete confluence.

To passage the cells, detach cells from culture vessel with Trypsin/EDTA, add complete growth medium and transfer to a tube, spin down cells, resuspend cells and seed appropriate aliquots of cells suspension into new culture vessels. Subcultivation ration = 1:10 to 1:20 weekly.To achieve satisfactory results, cells should not be passaged over 16 times.

Functional validation:

A. Response of AP-1 HEK293 cells to phorbol 12-myristate 13-acetate (PMA)

1. Harvest AP-1 HEK293 cells and seed cells into a white solid-bottom 96-well microplate in 100 µl of growth medium at 5 x 104 cells/well.
2. Incubate cells at 37°C in a CO2 incubator for overnight.
3. The next day, stimulate cells with different concentrations of PMA.
4. Incubate at 37°C in a CO2 incubator for 6-16 hours.
5. Add 50 µl of luciferase assay reagent per well.
6. Incubate at room temperature for 1-5 minutes and measure luminescence using a microplate luminometer.

AP-1 Luciferase Reporter-HEK293 Cell Line (RC1002) Images

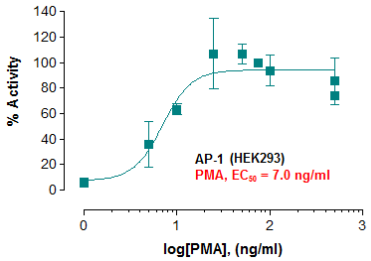


Fig-1: Induction of AP-1 activity by phorbol 12-myristate 13-acetate in AP-1 HEK293 cells.

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