

14-118ACL: HRE Lleeporter™ Luciferase Reporter-HeLa Cell Line

Application : Functional Assay

Description

The HRE Lleeporter™ Luciferase Reporter cell line is a stably transfected HeLa cell line which expresses Renilla luciferase reporter gene under the transcriptional control of the hypoxia response element (HRE). In response to hypoxia (low oxygen), HREs of target genes are recognized and regulated by the hypoxia-inducible factors (HIFs) which belong to the family of basic helix-loop-helix transcription factors and form heterodimeric complex comprising the alpha subunit (HIF-1 alpha, HIF-2 alpha and HIF-3 alpha) and beta subunit (Arnt1, Arnt2 and Arnt3), among which HIF-1 alpha and HIF-2 alpha are predominant isoforms. Activation of HIFs can also be mediated by chemical hydroxylase inhibitors as hypoxia mimetics including the iron chelator desferrioxamine and cobalt chloride. The HRE induction by cobalt chloride is shown in Figure 1.

Product Info

Amount : 1 vial
Content : Each vial contains $2 \sim 3 \times 10^6$ cells in 1 ml of 90% FBS + 10% DMSO.
Storage condition : Immediately upon receipt, store in liquid nitrogen.

Application Note

Application:

- Monitor the HIF induction activity.
- Screen for activators or inhibitors of the hypoxia signaling pathway.

Culture conditions:

Cells should be grown at 37°C with 5% CO₂ using DMEM medium (w/ L-Glutamine, 4.5g/L Glucose and Sodium Pyruvate) supplemented with 10% heat-inactivated FBS and 1% Pen/Strep, plus 2 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 1~3 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 HRE Looporter™ - HeLa cells to cobalt chloride (CoCl₂).

1. Harvest HRE Looporter™ - HeLa cells and seed cells into a white solid-bottom 96-well microplate in 100 µl of growth medium at 5×10^4 cells/well.
2. Incubate cells at 37°C in a CO₂ incubator for 6-16 hours.
3. The next day, stimulate cells with various concentrations of CoCl₂.
4. Incubate at 37°C in a CO₂ incubator for 16 hours.
5. Equilibrate the plate to room temperature for 10 minutes.
6. Add 50 µl of luciferase assay reagent (Abeomics, Cat #17-1101; Refer to the reagent datasheet for the detailed luciferase assay protocol) per well.
7. Read the plate in 1-5 minutes to measure luminescence using a microplate luminometer.

LIMITED USE RESTRICTIONS:

THIS PRODUCT IS SOLELY FOR IN VITRO RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

By use of this product, user agrees to be bound by the terms of this limited use statement.

This product is solely for Internal Research Purposes and not for Commercial Purposes. Commercial Purposes include, but are not limited to (1) use of the cell line in manufacturing; (2) use of the cell line to provide a service, information or data; (3) use of the cell line for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the cell line whether or not such cell lines are resold for use in research. The buyer cannot sell, give or otherwise transfer this product to a third party.

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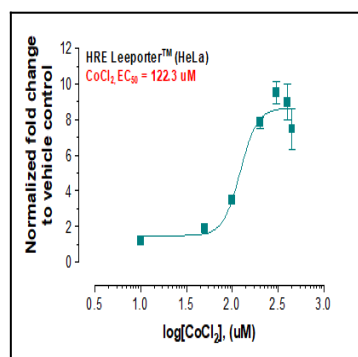


Fig-1: Induction of HRE activity by cobalt chloride in HRE Looporter™ - HeLa cells.