Retro-iPS Human Inducers

DATA SHEET

CAT# ABP-SC-RHOSKM4 *Retroviral vector plasmids with human Oct3/4, Sox2, KLF4, and c-Myc:*

· Storage conditions: -20°C

Background:

Induced pluripotent stem (iPS) cells were first reported by Takahashi et al., who used retroviral vector to transduce cDNAs selected from 24 mouse candidate genes that were shown to contribute to maintenance of the ES cell phenotype [1]. Only Four genes, Oct3/4, Sox2, Klf4, and c-Myc, were shown to be necessary to induce adult cells to become pluripotent to give rise to cell lineages of all 3 germ layers. Human homologues of these four genes were shown to induce human fibroblasts into iPS cells [2], and further, an alternative set of 4 human cDNAs consisting of Oct4 (aka POU5F1), Sox2 and Nanog, Lin28, with the latter two replacing Klf4 and c-Myc in the original Takahashi publication, were also able to generate human iPS from primary fibroblasts [3].
Allele Retro-iPS inducer plasmids include all six currently known human iPS inducer genes on the vector Gryphon vector pCHAC, which enables the researcher to generate recombinant retrovirus using Allele's Gryphon™ system, one of the most time-tested retrovirus systems in use over 3,000 laboratories worldwide, for gene delivery.

Description:

Allele Retro-iPS Human Inducer constructs, encoding six human cDNAs, were designed to help researchers to quickly and effectively initiate research in the cutting-edge iPS field. With the original sets of cDNAs on proven retroviral vectors, any lab capable of handling lab strain retrovirus can immediately proceed to create their own iPS cells.

Features:

· All 6 cDNAs published by the landmark iPS papers
· Ready to use for virus preparation
· Carried on one of the most popular retroviral vector system already widely adapted
· Packaging cells and custom services for viral preparation offered by Allele Biotech
· Easy switch to fluorescent protein and RNAi carrying viruses within the same product group

Applications:

Suitable for inducing adult human cells into iPS cells.

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium

Quality Assurance:
The plasmids have been sequence-confirmed and each batch is vigorously tested.

Reagents Provided with the Kit:
Plasmid with human cDNAs on pCHAC vectors, 10 ug each.

Recommended Protocols:
Use of Gryphon™ ampho packaging cells
And Gry™ eco packaging cells is recommended for transfection. Both are available through Allele Biotech™.

Day 0: Preparation of Producer cells for transfection:
·18-24 hours prior to transfection, seed Phoenix™ cells at 1.5-2 x 106 cells per 60 mm plate in GM.
·After adding cells gently shake 3-4 times to distribute cells evenly about the plate.
·Set plates in a 37ºC incubator. Subconfluent cells (60-70%) are best suited for transfection. Transfection efficiency of 50-60% should be achieved.

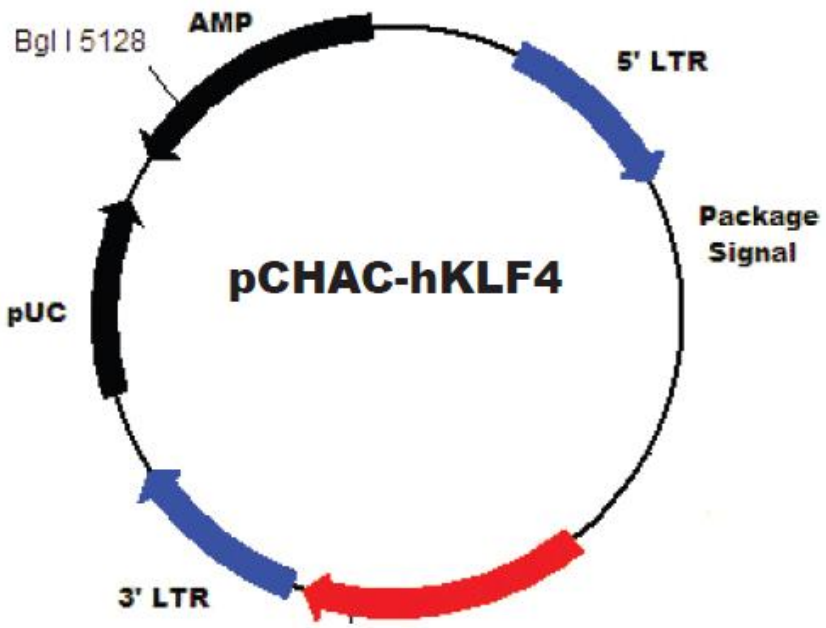
Day 1: Transfection:
·Transfect subconfluent Gryphon™ cells using either conventional cationic lipids or calcium phosphate protocols.
·Apply about 5-10 µg of plasmid DNA (pCHAC Inducer) per 60 mm dish. Including chloroquine (25 mM chloroquine stock solution prepared in either PBS or GM and filtered through a 0.2 µM filter and stored at -20°C) at 25 µM during calcium phosphate transfection have been found to yield higher viral titer (2 folds).
·After transfection, cells should be incubated at 32 ºC for greater virus stability and thus, higher yield. A lower level of viral production will occur at 37 ºC.
Day 2: 24 hours post-transfection: ·Overnight media should be replaced with 3 ml of fresh GM if treated with chloroquine for more than 24 hours

Day 3: Harvest at 48 hours post-transfection: ·Harvest supernatant from transfected Gryphon cells into 15 ml tubes and centrifuge at 2000 x g for 5 minutes to pellet cell debris. ·The virus stock can be frozen at -80 ºC for future use although the titer drops by one- half for each freeze-thaw cycle and thus, the virus stock should be stored in many small aliquots. **For more detailed transfection and Virus Titer protocol refer to Gryphon™ Retrovirus Expression System Protocol.*
***Allele Biotech™ does not recommend the use of ultracentrifugation to concentrate the retrovirus stock since hydrodynamic force generated during centrifugation can remove most of the viral glycoprotein and thus, the infectivity of virus particles. Transfection efficiency of transfected Gryphon ™ cells should be determined and optimized since this parameter is the primary determinant of high virus yield.*

References: 1. Takahashi, K. and S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic Cell, 2006. 126(4): p. 663-76. 2. Takahashi, K., et al., Induction of pluripotent stem cells from adult human 131(5): p. 861-72. 3. Yu, J., et al., Induced pluripotent stem cell lines derived from human somatic cells. Science, 2007. 318(5858): p. 1917-20.

Plasmid Maps:

ABP-SC-VCREKLF



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