Adeno-hTERT

Store at -80℃

Cat. No.	Description	Quantity
G205	AdhTERT	250ul

Description

The recombinant adenoviral vectors have been used to transduce many mammalian cell types (both dividing and non-dividing) for efficient gene expression of the recombinant proteins. Recombinant adenoviruses are especially useful for gene transfer and protein expression in cell lines that have low transfection efficiency with other transfection reagents. After entering cells, the virus remains epichromosomal (i.e. does not integrate into the host chromosome so does not activate or inactivate host genes). Recently, recombinant adenoviruses have been used to deliver RNAi into cells for specific gene knock-down.

Telomeres are specialised DNA repeats that stabilise the ends of chromosomes (1,2). As somatic cells divide, their telomeres gradually become shorter. At a critical telomere length, somatic cells enter into a nondividing state termed senescence (3). In contrast, germline cells have an extended life span and do not undergo senescence. Unlike somatic cells, germline cells actively express telomerase, which maintains the length of their telomeres.

Studies have demonstrated that normal primary cells could be immortalized by stable transfection with hTERT. Though hTERT immortlised cells can divide indefinitely, they maintain a primary cell phenotype, contact inhibition and serum deprivation. Additional experiments showed that telomerase-immortalised cells neither grow in soft agar nor form tumors in nude mice (7).

Adeno-hTERT is a recombinant telomerase adenovirus that is used to introduce Telomerase into any human (lower efficiency for blood) cells quickly and with the highest efficiency (100%), including primary cells. Expression of hTERT gene may immortalised cells transduced.

Cell Immortalisation Guidelines

1. Based on the in vitro growth patterns, there are essentially two types of primary cells: one that can be cultured for 20-50 passages before senescence; and one that can only be passed for less than 10 passages in culture conditions.
2. Cells that have a life span of 20-50 passages in vitro culture conditions include mostly blast cells such as fibroblasts and retinoblasts.
3. Cells that have less than 10 passages under in vitro culture conditions are mostly epithelial cells such as breast and ovarian epithelial cells.
4. To immortalise your cells, you can use either hTERT or SV40 T antigens for cells that can be cultured for over 10 passages.
5. It has been showed that introduction of hTERT may induce apoptosis in primary epithelial cells or other cells that have <10 passages of life span. It is recommended to use SV40 T antigen for those cells. In many epithelial cells, EGF has been shown to be able to increase their life span to 10 to 20 passages before senescence. So, you may try to add some recombinant EGF (10ng/ml) to expand your cell life span before hTERT gene transduction.
6. Cell immortalisation is a very complicated in vivo cellular process. Successful immortalisation with our products may vary from one cell type to anther

Protocol

1.When you receive your recombinant adenovirus, make 2-3 aliquots and use one for amplification in 293 cells. Freeze the other one or two aliquots in -70oC as seed stock for future use.
2. Amplify your Adenovector in 293 cells by infecting 293 cells with 10ul for 60mm dish, 200ul for 100mm dish.
Note: always amplify one adenoviral vector a time and amplify in different culture hood and incubator. Or do it sequentially if you just have one set of equipment in your lab and UV radiation for 30 minutes in between. Use separate trypsin and medium containers for each virus. Cross-contamination when working with two or more adenovectors is more common than you think. Once it occurred, your results will be greatly compromised. After you have got your adeno viral stock, you can work with 2 or more Adenovector safely in your targeting cells.
3.After more 95% 293 cells detached from dishes, collect both cells and medium.
4. Freeze (-70C freezer or dry ice/ethanol) and thaw (37oC water bath) 3 times.
5.Spin at 3,000 rpm RT to remove cell debries.
6. Transfer supernatant into a new tube. Store at 4oC for short-term use (2-3 weeks) or add glycerol (final concentration 10%) to it and freeze at –70oC (stable for 1-2 years).
7. Analysing your gene expression by Western blotting, Q-PCR, or under microscope if your gene of interest is a reporter gene such as b-gal or EGFP.
Note: you need to do large-scale virus production and purification for most in vitro gene transduction and do so when you are planning to do in vivo injection, which require much higher and purer adenovirus preparation.
8. For any further questions, drop us a note at info@nbsbio.co.uk

CERTIFICATE OF ANAYLSIS