

Expression Arrest™ β -gal Control Vector



RHS3708

Product Description:

The β -gal Control Vector contains β -gal under the control of the cytomegalovirus (CMV) immediate early promoter for high levels of protein expression in mammalian cells. Selection in bacteria is made possible by the inclusion of the ampicillin resistance marker bla. β -gal, the protein product of the e.coli *lacZ* gene, is a commonly used reporter since it is easy to use and can be assayed either histochemically or quantified using a spectrophotometer. Through the hydrolysis of X-gal, a blue precipitate can be visualized which allows the determination of transfection efficiency by calculating the ratio of blue (transfected) vs. non-transfected cells. Activity can also be quantified using a spectrophotometer following the addition of a substrate to produce a colored product (e.g. ONPG).

Contents:

10 μ g (0.5 μ g/ml) plasmid DNA resuspended in Tris buffer (pH 8.5)

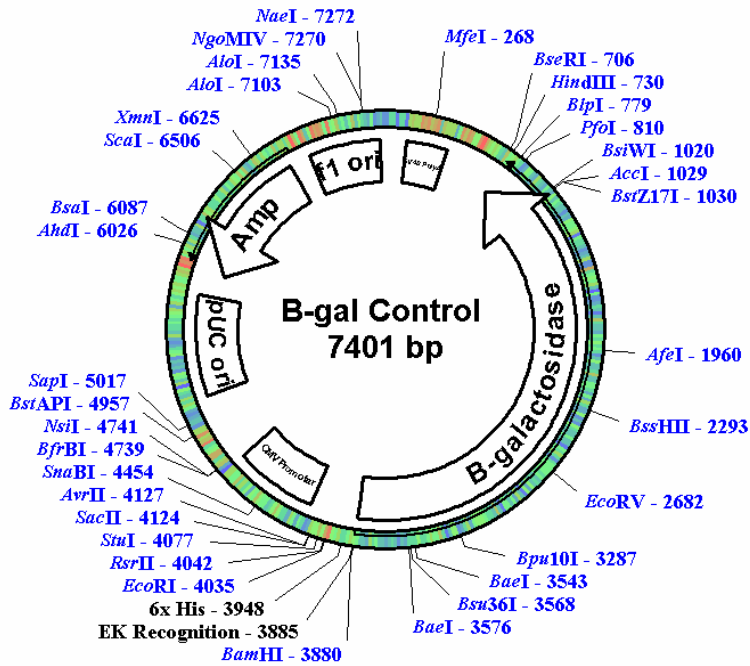
Quality Control:

High purity (≥ 1.9 ratio 260/280 Abs) transfection ready plasmid DNA was prepared and resuspended in Tris buffer (pH 8.5). Following plasmid DNA preparation the β -gal Control Vector was used in a transient transfection to verify β -galactosidase expression and its use as a control for transfection efficiency.

Storage:

Store at -20°C

Vector Map¹:



¹ Vector map and sequence compiled from literature and known fragments used to construct vector. Sequence information available at http://www.openbiosystems.com/product_inserts.php

Sequence:

1 CCATTGCGCA TTCAGGCTGC GCAACTGTTG GGAAGGGCGA TCGGTGCGGG
51 CCTCTTCGCT ATTACGCCAG CCAATACGCA AACCGCCTCT CCCCgcgCGT
101 TGGCCGATTC ATTAATGCAG GATCGATCCA GACATGATAA GATACATTGA
151 TGAGTTTGGG CAAACCACAA CTAGAATGCA GTGAAAAAAA TGCTTTATTT
201 GTGAAATTTG TGATGCTATT GCTTTATTTG TAACCATTAT AAGCTGCAAT
251 AAACAAGTTA ACAACAACAA TTGCATTCAT TTTATGTTTC AGGTTTCAGGG
301 GGAGGTGTGG GAGGTTTTTT AAAGCAAGTA AAACCTCTAC AAATGTGGTA
351 TGGCTGATTA TGATCATGAA CAGACTGTGA GGACTGAGGG GCCTGAAATG
401 AGCCTTGGGA CTGTGAATCT AAAATACACA AACAATTAGA ATCACTAGCT
451 CCTGTGTATA ATATTTTCAT AAATCATACT CAGTAAGCAA AACTCTCAAG
501 CAGCAAGCAT ATGCAGCTAG TTTAACACAT TATACTACTA AAAATTTTAT
551 ATTTACCTTA GAGCTTTAAA TCTCTGTAGG TAGTTTGTCC AATTATGTCA
601 CACCACAGAA GTAAGGTTCC TTCACAAAGA TCCCAAGCTA GCAGTTTTCC
651 CAGTCACGAC GTTGTA AAC GACGGCCAGT GCCTAGCTTA TAATACGACT
701 CACTATAGGG ACCACTCCTC GATACGCGTA AGCTTTTATT TTTGACACCA
751 GACCAACTGG TAATGGTAGC GACCGGCGCT CAGCTGTAAT TCCGCCGATA
801 CTGACGGGCT CCAGGAGTCG TCGCCACCAA TCCCCATATG GAAACCGTCG
851 ATATTCAGCC ATGTGCCTTC TTCCGCGTGC AGCAGATGGC GATGGCTGGT
901 TTCCATCAGT TGCTGTTGAC TGTAGCGGCT GATGTTGAAC TGGAAGTCGC
951 CGCGCCACTG GTGTGGGCCA TAATTCAATT CGCGCGTCCC GCAGCGCAGA
1001 CCGTTTTTCG TCGGGAAGAC GTACGGGGTA TACATGTCTG ACAATGGCAG
1051 ATCCCAGCGG TCAAAACAGG CGGCAGTAAG GCGGTCGGGA TAGTTTTCTT
1101 GCGGCCCTAA TCCGAGCCAG TTTACCCGCT CTGCTACCTG CGCCAGCTGG
1151 CAGTTCAGGC CAATCCGCGC CGGATGCGGT GTATCGCTCG CCACTTCAAC
1201 ATCAACGGTA ATCGCCATTT GACCACTACC ATCAATCCGG TAGGTTTTCC
1251 GGCTGATAAA TAAGGTTTTT CCCTGATGCT GCCACGCGTG AGCGGTCGTA
1301 ATCAGCACCG CATCAGCAAG TGTATCTGCC GTGCACTGCA ACAACGCTGC
1351 TTCGGCCTGG TAATGGCCCC CCGCCTTCCA GCGTTCGACC CAGGCGTTAG
1401 GGTCAATGCG GGTGCTTCA CTTACGCCAA TGTCGTTATC CAGCGGTGCA
1451 CGGGTGAACT GATCGCGCAG CGGCGTCAGC AGTTGTTTTT TATCGCCAAT
1501 CCACATCTGT GAAAGAAAAG CTGACTGGCG GTTAAATTGC CAACGCTTAT
1551 TACCAGCTC GATGCAAAA TCCATTTTCG TGGTGGTCAG ATGCGGGATG
1601 GCGTGGGACG CGGCGGGGAG GTCACACTG AGGTTTTCCG CCAGACGCCA
1651 CTGCTGCCAG GCGCTGATGT GCCCGGCTTC TGACCATGCG GTCGCGTTCCG
1701 GTTGCACTAC GCGTACTGTG AGCCAGAGTT GCCCGGCGCT CTCCGGCTGC
1751 GGTAGTTCAG GCAGTTCAAT CAACTGTTTA CCTTGTGGAG CGACATCCAG
1801 AGGCACTTCA CCGCTTGCCA GCGGCTTACC ATCCAGCGCC ACCATCCAGT
1851 GCAGGAGCTC GTTATCGCTA TGACGGAACA GGTATTGCTG GGTCACTTCG
1901 ATGGTTTGCC CGGATAAACG GAACTGGAAA AACTGCTGCT GGTGTTTTGC
1951 TTCCGTCAGC GCTGGATGCG GCGTGCGGTC GGCAAAGACC AGACCGTTCA
2001 TACAGAAGT GCGATCGTTC GCGGTATCGC CAAAATCACC GCCGTAAGCC
2051 GACCACGGGT TGCCGTTTTT ATCATATTTA ATCAGCGACT GATCCACCCA
2101 GTCCAGACG AAGCCGCCCT GTAAACGGGG ATACTGACGA AACGCCTGCC
2151 AGTATTTAGC GAAACCGCCA AGACTGTTAC CCATCGCGTG GCGTATTTCG
2201 CAAAGGATCA GCGGGCGCGT CTCTCCAGGT AGCGAAAGCC ATTTTTTGAT
2251 GGACCATTTT GGCACAGCCG GGAAGGGCTG GTCTTCATCC ACGCGCGCGT
2301 ACATCGGGCA AATAATATCG GTGGCCGTGG TGTCGGCTCC GCCGCTTCA
2351 TACTGCACCG GCGGGGAAGG ATCGACAGAT TTGATCCAGC GATACAGCGC
2401 GTCCTGATTA GCGCCGTGGC CTGATTCATT CCCCAGCGAC CAGATGATCA
2451 CACTCGGGT ATTACGATCG CGCTGCACCA TTCGCGTTAC GCGTTGCTC
2501 ATCGCCGGTA GCCAGCGCGG ATCATCGGTC AGACGATTCA TTGGCACCAT
2551 GCCGTGGGTT TCAATATTGG CTTTCATCCAC CACATACAGG CCGTAGCGGT
2601 CGCACAGCGT GTACCACAGC GGATGGTTTC GATAATGCGA ACAGCGCACG
2651 GCGTTAAAGT TGTTCTGCTT CATCAGCAGG ATATCCTGCA CCATCGTCTG

2701 CTCATCCATG ACCTGACCAT GCAGAGGATG ATGCTCGTGA CGGTTAACGC
2751 CTCGAATCAG CAACGGCTTG CCGTTCAGCA GCAGCAGACC ATTTTCAATC
2801 CGCACCTCGC GGAAACCGAC ATCGCAGGCT TCTGCTTCAA TCAGCGTGCC
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2951 GCATAACCAC CACGCTCATC GATAATTTCA CCGCCGAAAG GCGCGGTGCC
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4151 ATCGTCCCG GTGTCTTCTA TGGAGGTCAA AACAGCGTGG ATGGCGTCTC
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4251 CCGTACACGC CTACCGCCCA TTTGCGTCAA TGGGGCGGAG TTGTTACGAC
4301 ATTTTGAAA GTCCC GTTGA TTTTGGTGCC AAAACAAACT CCCATTGACG
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4851 CCCCTATTTG TTTATTTTTT TAAATACATT CAAATATGTA TCCGCTCATG
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7251 TTAGAGCTTG ACGGGGAAAG CCGGCGAACG TGGCGAGAAA GGAAGGGAAG
7301 AAAGCGAAAG GAGCGGGCGC TAGGGCGCTG GCAAGTGTAG CGGTACGCT
7351 GCGCGTAACC ACCACACCCG CCGCGCTTAA TGCGCCGCTA CAGGGCGCGT
7401 C

Co-Transfection of Adherent Cells with Reporters and shRNA constructs

****Warm Arrest-In™ reagent to ambient temperature (approximately 10-15 minutes at room temperature) prior to use. Always mix well by vortex or inversion prior to use. Do not add antibiotics to cell media during transfection**

1. The day before transfection, plate the cells so that they are approximately 70-80% confluent on the day of transfection.
2. The protocol below is optimized for co-transfection of a reporter and a shRNA construct into Hek293T cells in a 24-well plate ($2.8\text{-}3 \times 10^4$ cells per well seeded the day before transfection). If a different culture dish is used adjust all amounts in proportion to the change in surface area. Form shRNA pDNA/Arrest-In™ transfection complexes as follows:
 - a. For each well in transfection, in a sterile microcentrifuge tube dilute into 40 μl serum free medium the following:
 - i. 50ng of the reporter DNA to be targeted by the shRNA
 - ii. 20ng of a non-targeted reporter, to be used as a transfection efficiency control (e.g. B-gal)
 - iii. 500ng of the appropriate shRNA plasmid DNA
 - iv. Add serum free medium as required to bring the final volume to 50ul
 - b. For each well in transfection, in a sterile microcentrifuge tube dilute 2.85 μl (2.85 μg) Arrest-In™ into 48 μl serum free medium.
 - c. Add the 50 μl of medium containing the diluted DNA (step a) to the diluted Arrest-In™ (step b), mix and incubate for 15 minutes to form the transfection complexes.
3. Add the transfection complex mixture to the cells and incubate in a CO₂ incubator at 37°C.
 - a. Method 1 – Add the transfection complex mixture directly to the cells in culture. Tap the plate and shake carefully to mix. Return the cells to a CO₂ incubator at 37°C. The following day replace medium with fresh growth medium as required.
 - b. Method 2 – Serum free transfection for difficult to transfect lines, to increase efficiency, or sensitive cell lines. First aspirate the growth medium from the cells. Add an additional 300ul of serum free medium to the tube containing the transfection complexes, mix well, then overlay onto the cells. Return the cells to the CO₂ incubator at 37°C for 3-6 hrs. Following the incubation add an equal volume of growth medium containing twice the amount of normal serum to the cells (i.e. to bring the overall concentration of serum to what is typical for your cell line). Alternatively the transfection medium can be aspirated and replaced with

the standard culture medium (see Note). Return the cells to the CO₂ incubator at 37°C.

Note – Arrest-In™ has displayed low toxicity in the cell lines tested therefore removal of transfection reagent is not required for many cell lines. In our hands higher transfection efficiencies have been achieved if the transfection medium is not removed. However, if toxicity is a problem, aspirate the transfection mixture after 3-6 hrs and replace with fresh growth medium. Additionally, fresh growth medium should be replenished as required for continued cell growth

4. After 48 hrs of incubation, assay cells for reduction in gene activity, compared to reporter alone, Non-silencing shRNA, or other negative controls.
5. Calculate decrease in reporter activity compared to % of control as follows:
 - a. Calculate the mean of your replica samples (R1 + R2...) of shRNA/reporter transfections and the Non-silencing/reporter or other negative controls.
 - b. Subtract the mean of a 'no plasmid DNA' control (background) from each sample
 - c. Divide each of the background subtracted replica means by the mean of the Non-silencing/Reporter transfections (or other negative control transfection). This results in your sample:control ratio.
 - d. Assay for the non-targeted reporter activity (e.g. B-gal, Renilla Luciferase) to determine if transfection efficiency varied across wells. If significant variation exists either repeat transfection under tightly controlled conditions or normalize transfection efficiency prior to comparing across wells.

Note - Factors affecting transfection efficiency are not limited to but include purity of plasmid DNA, health of transfected cells, inconsistencies in number of cells plated, insufficient mixing of transfection complexes.

Additional Factors Influencing Successful Transfection:

1. Concentration and purity of nucleic acids – Determine the concentration of your DNA using 260 nm absorbance. Avoid cytotoxic effects by using pure preparations of nucleic acids.
2. Transfection in serum containing or serum-free media – Our studies indicate that Arrest-In™ /DNA complexes should always be formed in the absence of serum. In the cell lines tested we found that the highest transfection efficiencies can be obtained if the cells are exposed to the transfection complexes in serum free conditions followed by the addition of medium containing twice the amount of normal serum to the complex medium 3-5 hrs post transfection (leaving the complexes on the cells). However, the transfection medium can be replaced with

- normal growth medium if high toxicity is observed.
3. Presence of antibiotics in transfection medium – The presence of antibiotics can adversely affect the transfection efficiency and lead to increased toxicity levels in some cell types. It is recommended that these additives be initially excluded until optimized conditions are achieved, then these components can be added, and the cells can be monitored for any changes in the transfection results.
 4. High protein expression levels – Some proteins when expressed at high levels can be cytotoxic, this effect can also be cell line specific.
 5. Cell history, density, and passage number – It is very important to use healthy cells that are regularly passaged and in growth phase. The highest transfection efficiencies are achieved if cells are plated the day before, however, adequate time should be allowed to allow the cells to recover from the passaging (generally >12 hours). Plate cells at a consistent density to minimize experimental variation. If transfection efficiencies are low or reduction occurs over time thawing a new batch of cells or using cells with a lower passage number may improve the results.

Table 1 - Suggested amounts of DNA, medium and Arrest-In™ reagent for transfection of shRNA constructs into adherent cells

Tissue Culture Dish	Surface area per well (cm ²)	Total serum free media volume per well (ml)	shRNA pDNA (µg)*	Arrest-In™ (µg)**
60 mm	20	2	4	21
35 mm	8	1	2	10
6-well	9.4	1	2	10
12-well	3.8	0.5	1	5
24-well	1.9	0.25	0.5	2.5
96-well	0.3	0.1	0.1 - 0.2	0.5 - 1

*Recommended starting amount of DNA. May need to be optimized for the highest efficiency

**Recommended starting amounts of Arrest-In™ reagent. See Transfection Optimization.