pAM_1C Empty Vector

Catalog No.: 53023
Format: 20 µg

Description

The pAM_1C Empty Vector is designed for use with Active Motif's Tag-ChIP-IT® Kit (Catalog No. 53022). The DNA sequence of interest is cloned into the pAM_1C vector using the HindIII restriction site to append the AM-tag sequence to the carboxy-terminus of the protein of interest. Alternatively, the AM-tag coding region can be removed and transferred into other protein expression vectors as a C-terminus tag. pAM_1C contains the Human Beta-actin promoter (ACTB) which provides constitutive high-level protein expression in mammalian systems. The vector may be used for transient transfections, or puromycin selection can be applied to select for stable gene expression. Western blot analysis with the AM-Tag antibody (Catalog No. 61677) can confirm tagged-protein expression. Cells expressing the fusion protein are ready for use in the Tag-ChIP-IT Kit.

Contents

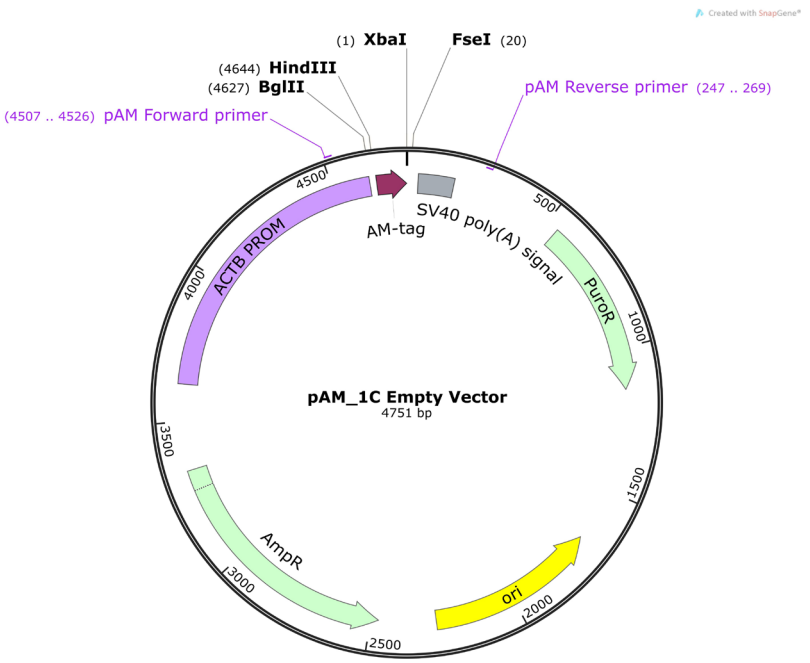
- 20 µg of pAM_1C Empty Vector provided as lyophilized DNA.
- 250 pmol pAM Forward primer for DNA sequencing provided as lyophilized DNA.
- 250 pmol pAM Reverse primer for DNA sequencing provided as lyophilized DNA.

Resuspend plasmid to a concentration of 1 µg/µl in sterile H_2O. Store reconstituted DNA at -20°C. Resuspend DNA sequencing primers to a concentration of 5 pmol/µl in sterile H_2O or TE Buffer (pH 7.5-8.0). Store reconstituted primers at -20°C.

pAM_1C Empty Vector Features and Circle Map

The following features are present in the pAM_1C Empty Vector based on nucleotide sequence.

SV40 poly(A) signal	40-161
pAM Reverse primer	247-269
Puromycin coding region	543-1142
SV40 poly(A) signal	1149-1283
*Col*E1-derived plasmid origin of replication	1686-2274
β-lactamase (Amp^r) coding region	2474-3334
Synthetic polyadenylation signal	3439-3487
RNA pol II transcriptional pause site	3501-3592
ACTB promoter	3627-4626
pAM Forward primer	4507-4526
Universal cloning site (HindIII)	4644
AM-tag	4650-4751



Note: The insert must contain an in-frame ATG codon for translation initiation.

pAM_1C contains a TAG stop codon immediately following the AM-tag sequence.

Quality Control

Plasmid construct has been confirmed by restriction analysis and sequence verified.

Shipping & Storage

Products are shipped at room temperature.
Lyophilized DNA is stable for 12 months when stored at -20°C.
Resuspended DNA is stable for 6 months when stored at -20°C. Avoid repeated freeze/thaw cycles.

GENERAL PRODUCT USE

pAM_1C is an expression plasmid containing the constitutive mammalian ACTB promoter, a HindIII restriction site for cloning and a C-terminal AM-tag. This vector may be used for cloning and expression of the gene of interest or as a template for cloning the AM-tag into another expression vector.

The following guidelines are provided for cloning:

1. Design a fusion protein construct containing the AM-tag in-frame with the C-terminus of the protein of interest. We recommend the use of directional cloning methods such as InFusion® (Clontech) or Gibson Assembly® (New England BioLabs). Follow the manufacturer's recommendations for cloning.

   Alternatively, the AM-tag sequence can be cloned into the expression vector of choice for your protein of interest. Ensure that the open reading frame of interest begins with the ATG start codon, that the AM-tag remains in-frame with the C-terminus and that a stop codon is present. We do not recommend the use of the AM-tag at the N-terminus as this may significantly reduce expression efficiency. If you require a N-terminal tag, please contact Active Motif's technical support.
2. Perform transformations of the AM-tag fusion protein construct into competent *E.coli*. We recommend the use of Active Motif's Rapid Trans TAM1 Competent cells (Catalog No. 11096) or other commonly used *E.coli* strains such as DH5a. Use the appropriate antibiotic selection for the cloning vector chosen (*e.g.* pAM_1C contains the ampicillin resistance gene).
3. Select 2-4 colonies for each construct and isolate the plasmid DNA. Sequence validate each plasmid to confirm the correct open reading frame sequence. We suggest preparing a glycerol stock for each candidate construct.
4. Determine the amount of DNA needed for transfection. If necessary, prepare additional DNA. Ensure that the DNA is free of nucleases or other contaminants prior to transfection. Quantify the DNA.

Following sequence validation of the AM-tag cloning vector, cells can be transiently transfected or selected to make stable cells expressing the AM-tagged protein of interest. Chromatin will be prepared from these cells for analysis in the Tag-ChIP-IT Kit (Catalog No. 53022). Successful chromatin immunoprecipitation depends on the efficiency of the transfection and the expression levels of the AM-tag fusion protein of interest. It is recommended to optimize the transfection and expression of tagged protein prior to performing ChIP.

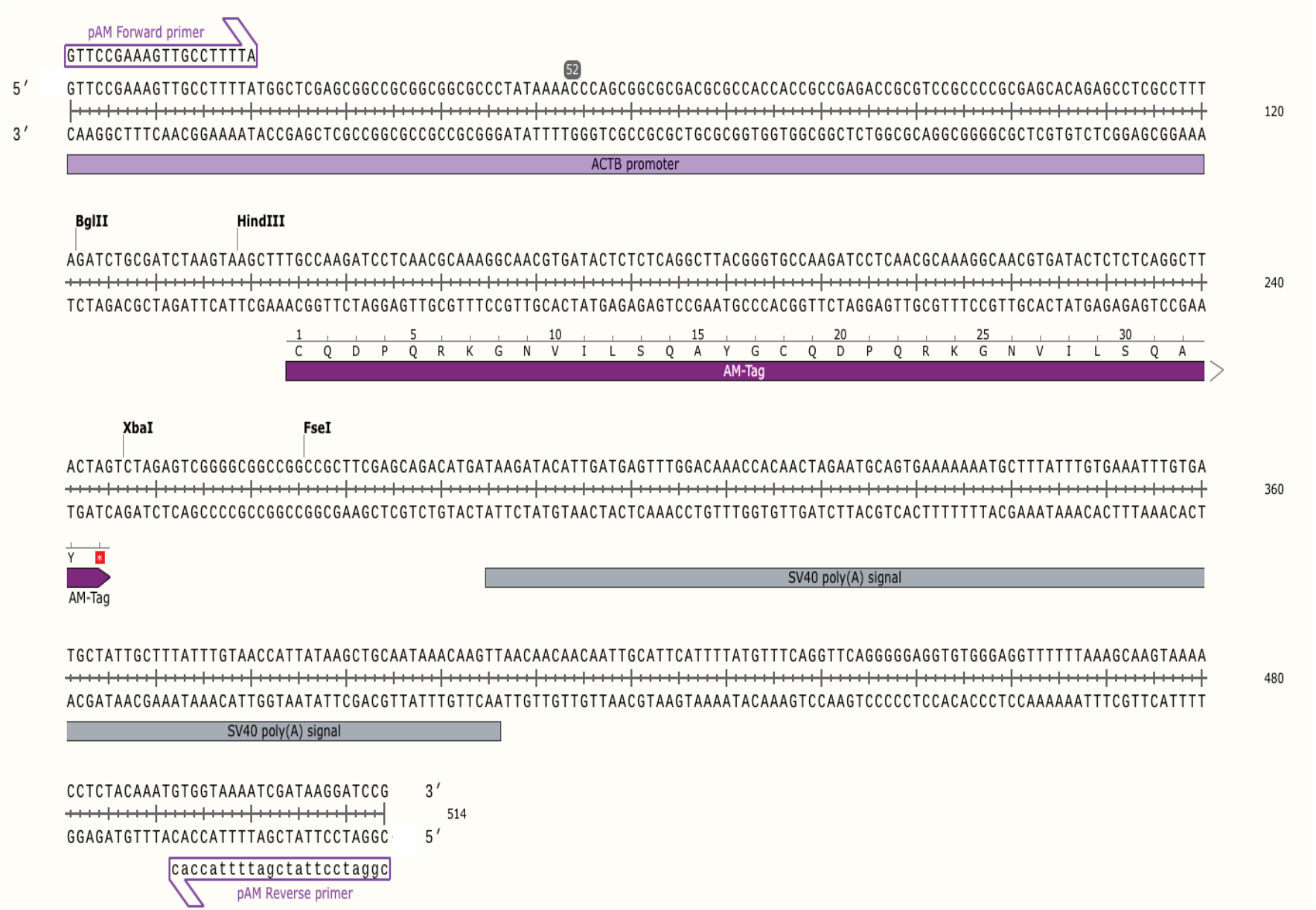
Once transfection conditions have been optimized, calculate the number of chromatin preparations you will require and determine the number of ChIP reactions you plan to perform on each chromatin preparation. Be sure to include the appropriate control ChIP reactions in your calculations. We recommend using untransfected cells (or a non-expressing cell line) as an estimate of the background. It is important to transfect enough cells to yield the necessary 20-30 µg of chromatin per immunoprecipitation reaction.

We recommend using FuGENE® HD Transfection Reagent (Catalog No. 32042) with the pAM_1C Vector at a ratio of 3:1 (µl FuGENE : µg DNA vector). Optimization may be required for each cell line and construct tested.

The following guidelines are provided for transfection:

1. In a 100 mm dish, seed 2.2 x 10^6 cells per plate in the appropriate growth medium. Incubate in a humidified incubator for 24 hours.

   (Optional) Prepare transfection reactions to verify expression of the AM-tagged fusion protein. We suggest performing a time course experiment to evaluate tagged-protein expression levels at 24, 48 and 72 hours post-transfection to identify the optimal harvest time point for the protein of interest. Cell lysates can be prepared from these transfection reactions for analysis by Western blot. Use the AbFlex™ AM-Tag antibody (Catalog No. 91111) at a 1:500 - 1:1000 dilution for detection of the tagged protein as compared to untransfected cells.
2. Prepare a separate microcentrifuge tube for each transfection reaction. Add 10 µg AM-tag construct to Opti-MEM media in a final volume of 550 µl.
3. Add 30 µl FuGENE HD Transfection Reagent drop wise directly to the media/DNA mixture. Do not allow FuGENE to come directly into contact with the plastic. Mix the solution by pipetting up and down and incubate at room temperature for 15-30 minutes.
4. Add 580 µl of the media/DNA/FuGENE mixture drop wise to each 100 mm dish. Gently swirl dish or incubate on a shaker at 100 rpm for 2 minutes to evenly distribute transfection mixture.
5. Return dish to humidified incubator for 24-72 hours before proceeding with the chromatin preparation. If desired, treat cells with compounds.
6. Refer to the Tag-ChIP-IT manual to prepare chromatin for use in ChIP.



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