pAM_1C_JunD Vector

Catalog No.: 53044
Format: 50 µg

Description

The pAM_1C_JunD Vector is designed for use with Active Motif's Tag-ChIP-IT® Kit (Catalog No. 53022). The DNA sequence for transcription factor JunD (NCBI Accession NM_005354.5) was cloned into the pAM_1C empty vector using InFusion Cloning® (Clontech) to append the AM-tag sequence to the carboxy-terminus. pAM_1C_JunD 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.

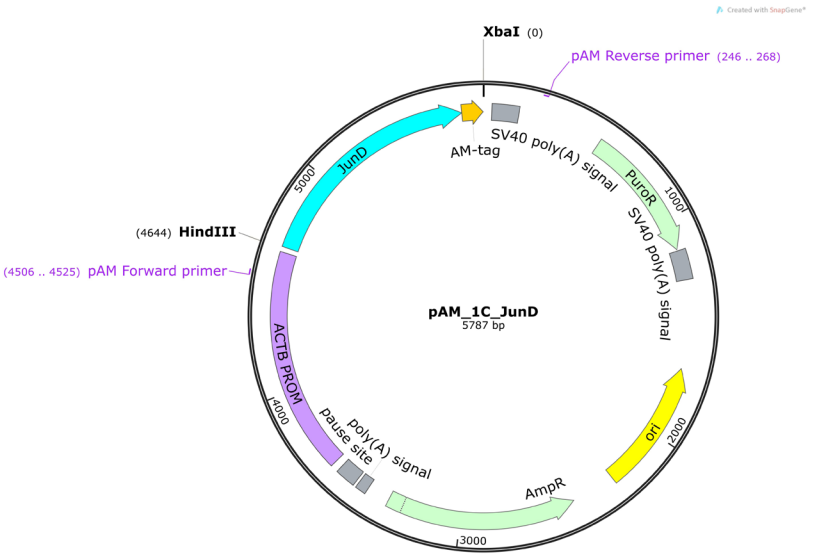
Contents

- 50 µg of pAM_1C_JunD Vector provided as lyophilized DNA.
  Resuspend plasmid to a concentration of 1 µg/µl in sterile H_2O. Store reconstituted DNA at -20°C.

pAM_1C_JunD Vector Features and Circle Map

The following features are present in the pAM_1C_JunD Vector based on nucleotide sequence.

SV40 poly(A) signal	39-160
pAM Reverse primer	246-268
Puromycin coding region	542-1141
SV40 poly(A) signal	1148-1282
*Col*E1-derived plasmid origin of replication	1685-2273
β-lactamase (Amp^r) coding region	2473-3333
Synthetic polyadenylation signal	3438-3486
RNA pol II transcriptional pause site	3500-3591
ACTB promoter	3626-4626
pAM Forward primer	4505-4525
JunD	4650-5690
AM-tag	5691-5786



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.

Background

JunD is a component of the activator protein-1 (AP-1) transcription factor family. AP-1 proteins play a role in the expression of many genes involved in proliferation and cell cycle progression including neuronal apoptosis, learning process, drug-induced behavioral responses, bone growth and differentiation, and embryo development. AP-1 is composed of a mixture of heterodimeric complexes of proteins derived from the Fos and Jun families including c-Fos, FosB, Fra-1, Fra-2, c-Jun, JunB and JunD. Only Jun proteins can form transcriptionally active homodimers. The N-terminus of Jun proteins is involved in transcriptional activation, while the C-terminus is involved in dimerization and DNA binding. AP-1 dimers bind the TPA-response element (TRE). JunD exists as two distinct isoforms (40 kDa & 45 kDa) generated by the use of two translational start sites within the JunD mRNA. Full length JunD contains an additional 48 amino acids at the amino-terminus. Both isoforms are expressed in all cell types at approximately the same levels.

GENERAL PRODUCT USE

pAM_1C_JunD can be used as a control vector in Active Motif's Tag-ChIP-IT® Kit (Catalog No. 53022). We have validated this vector using the following transfection conditions in HCT116 cells (human colorectal carcinoma). Optimization of transfection conditions may be required for use in other cell types.

1. In a 100 mm dish, seed 2.2 x 10^6 HCT116 cells per dish in McCoy's 5A medium supplemented with 10% fetal bovine serum. Incubate in a humidified incubator for 24 hours. We recommend setting up two dishes. One dish can be used for chromatin preparation and the second dish can be used to confirm expression of the tagged protein by Western blot.
2. Prepare a separate microcentrifuge tube for each transfection reaction. Add 10 µg pAM_1C_JunD construct to Opti-MEM media in a final volume of 550 µl.
3. Add 30 µl FuGENE HD Transfection Reagent (Catalog No. 32042) 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 48 hours before proceeding with the chromatin preparation.
6. Refer to the Tag-ChIP-IT manual to prepare chromatin.
7. Use Active Motif's Nuclear Extract Kit (Catalog No. 40010) to prepare nuclear lysates from the second dish for detection of the tagged protein as compared to untransfected cells. Use 20 µg nuclear lysate denatured at 98C for 10 minutes in sample loading buffer per well of a 4-20% Tris-Glycine gel. Run at 90 mAmps until the dye front runs off the gel. Transfer to a nitrocellulose membrane for 90 minutes at 35 volts. Block the membrane with 5% non-fat milk in PBS for 30 minutes. Add the AbFlex™ AM-Tag antibody (Catalog No. 91111) at a 1:500 - 1:1000 dilution in 5% non-fat milk in PBS for 1 hour. Wash the membrane three times for 5 minutes with distilled water. Add an anti-mouse HRP-conjugated secondary at 1:1000 dilution for 1 hour in 5% non-fat milk in PBS. Wash the membrane three times for 5 minutes with PBS containing 0.05% Tween 20 followed by 3-5 rinses of the membrane with distilled water. Detect with ECL or chemiluminescent substrate.

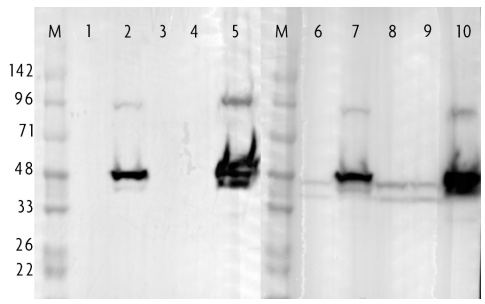


Figure 1: Validation of protein expression by WB.

Active Motif's pAM_1C_JunD vector was prepared containing the DNA sequence for transcription factor JunD in-frame with a C-terminal AM-tag. Hela and HCT 116 cells were seeded at 2.2 x 10^6 cells and transfected with 10 µg pAM_1C_JunD DNA, mock transfected or transfected with 10 µg pAM_1C Empty Vector. 48 hours post-transfection nuclear lysates were prepared using Active Motif's Nuclear Extract Kit (Catalog No. 40010). 20 µg lysate was loaded per lane.. AbFlex™ AM-Tag antibody (Catalog No. 91111) or JunD antibody were used at 1:500 dilution.

Lane M: Molecular weight marker (kDa)
Lane 1: Mock transfection in HeLa cells + 1:500 AM-Tag antibody
Lane 2: AM-Tag-JunD transfection in HeLa cells + 1:500 dilution AM-Tag antibody
Lane 3: Mock transfection in HCT116 cells + 1:500 AM-Tag antibody
Lane 4: Empty Vector transfection in HCT116 cells + 1:500 AM-Tag antibody
Lane 5: AM-Tag-JunD transfection in HCT116 cells + 1:500 dilution AM-Tag antibody
Lane 6: Mock transfection in HeLa cells + 1:500 JunD antibody
Lane 7: AM-Tag-JunD transfection in HeLa cells + 1:500 dilution JunD antibody
Lane 8: Mock transfection in HCT116 cells + 1:500 JunD antibody
Lane 9: Empty Vector transfection in HCT116 cells + 1:500 JunD antibody
Lane 10: AM-Tag-JunD transfection in HCT116 cells + 1:500 dilution JunD antibody

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

Active Motif North America
1914 Palomar Oaks Way, Suite 150
Carlsbad, CA 92008
USA
Toll Free: 877 222 9543
Telephone: 760 431 1263
Fax: 760 431 1351
E-mail: tech_service@activemotif.com

Active Motif Europe
Avenue Reine Astrid, 92
B-1330 La Hulpe, Belgium
UK Free Phone: 0800 169 31 47
France Free Phone: 0800 90 99 79
Germany Free Phone: 0800 181 99 10
Telephone: +32 (0)2 653 0001
Fax: +32 (0)2 653 0050
E-mail: eurotech@activemotif.com

Active Motif Japan
Azuma Bldg, 7th Floor
2-21 Ageba-Cho, Shinjuku-Ku
Tokyo, 162-0824, Japan
Telephone: +81 3 5225 3638
Fax: +81 3 5261 8733
E-mail: japantech@activemotif.com

Active Motif China
787 Kangqiao Road
Building 10, Suite 202, Pudong District
Shanghai, 201315, China
Telephone: (86)-21-20926090
Hotline: 400-018-8123

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