pAM_dCas9_CTCF Vector

Catalog No.: 53124
Format: 10 µg

Description

The pAM_dCas9_CTCF Vector is designed for use with Active Motif's enChIP Kit (Catalog No. 53125). The enChIP method relies on the CRISPR/Cas9 system to direct a guide RNA (gRNA) to a specific genomic locus for immunoprecipitation. The gRNA is designed as the complementary sequence of the desired target locus. An enzymatically inactive form of the *Streptococcus pyogenes* Cas9 protein, which contains Active Motif's unique AM-tag sequence, is co-expressed with the gRNA. The gRNA directs the dCas9 protein to its target sequence, immediately upstream of a Protospacer Adjacent Motif (PAM) (5´- NGG). Recognition of a PAM site leads to unwinding of the DNA and formation of an RNA-DNA heteroduplex. Cells are then formaldehyde fixed and chromatin is prepared. An antibody directed against the AM-tag is used to enrich for genomic sequences bound by the gRNA/dCas9 complex. DNA can be analyzed by qPCR or NGS to identify the enriched genomic regions. The pAM_dCas9_CTCF Vector can be used as a positive control for the enChIP Kit. It contains a gRNA sequence corresponding to a CTCF binding site on chromosome 19 (5,804,115-5,804,209) which has been cloned into the pAM_dCas9 empty vector (Catalog No. 53122). The pAM_dCas9_CTCF vector should be used with the single vector transfection protocol.

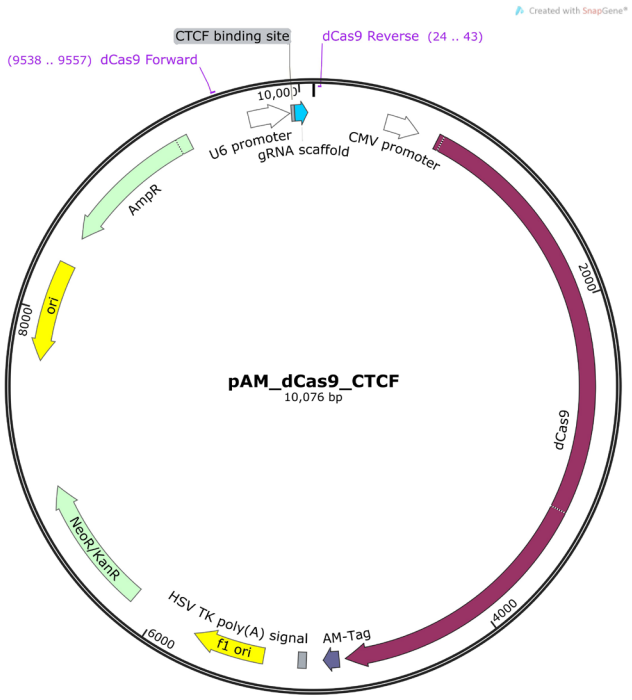
Contents

- 10 µg of pAM_dCas9_CTCF Vector provided at a concentration of 100 ng/µl.

pAM_dCas9_CTCF Vector Features and Circle Map

The following features are present in the pAM_dCas9_CTCF Vector based on nucleotide sequence.

dCas9 reverse primer	24-43
CMV promoter	430-633
dCas9 coding region	748-4851
AM-Tag	4888-4980
Herpes simplex virus TK poly (A) signal	5079-5127
f1 origin of replication	5329-5757
Neomycin^R/Kanamycin^R coding region	6167-6961
SV40 polyadenylation signal	7137-7258
*Col*E1-derived plasmid origin of replication	7709-8297
β-lactamase (Amp^r) coding region	8468-9328
dCas9 forward primer	9538-9557
U6 promoter	9699-9939
CTCF binding site	9949-9967
gRNA scaffold	9969-10044



Note: The pAM_dCas9 empty vector (Catalog No. 53122) is available separately and is recommended for use in combination with pAM_dCas9_CTCF as a no gRNA negative control.

Quality Control

Plasmid construct has been confirmed by restriction analysis and sequence verified. For the complete pAM_dCas9_CTCF Vector sequence, please visit the Documents tab at www.activemotif.com/enchip.

Shipping & Storage

Products are shipped on dry ice.
Resuspended DNA is stable for 6 months when stored at -20°C. Avoid repeated freeze/thaw cycles.

Transfection of pAM_dCas9_CTCF expression plasmid

Cells can be transiently transfected with the pAM_dCas9_CTCF expression plasmid. The following protocol provides recommendations for transfection using FuGENE® HD Transfection Reagent (Catalog No. 32042). Optimization may be required for each cell line tested.

To determine the efficiency of the transfection, set up a duplicate transfection for each cell type used. Cell lysates can be prepared from the duplicate transfection reaction using Active Motif's Nuclear Extract Kit (Catalog No. 40010) for analysis by Western blot. Use the AM-Tag polyclonal antibody (Catalog No. 61677) at a 1:250 - 1:1,000 dilution for detection of the AM-tagged dCas9 protein. If the tagged protein is not detected, continue to optimize transfection conditions.

Calculate the number of transfections you will perform. Small scale reactions provide enough chromatin to perform one enChIP reaction. Large scale reactions provide enough chromatin to perform 5 enChIP reactions. We recommend running a no gRNA negative control reaction (empty pAM_dCas9 vector, Catalog No. 53122) for each cell line tested.

1. Seed cells in either a 6-well plate (small scale) or 10cm dish (large scale) using the appropriate growth medium. Incubate in a humidified incubator for 24 hours. Cells should be 70-80% confluent at the time of transfection.

	Small Scale Cell Culture (1 enChIP rxn) Single Vector	Large Scale Cell Culture (5 enChIP rxns) Single Vector
Cell culture plate	6-well plate	10 cm dish
Cell seeding density*	5 x 10^5 cells	3 x 10^6 cells
Growth medium	10 ml	20 ml

*These conditions were established for cells with doubling times of 14-18 hours. Cell seeding densities may need to be optimized to ensure that cells are ~80% confluent at the time of transfection.

2. Prepare a separate microcentrifuge tube for each transfection reaction. To each tube add the recommended amount of DNA and Opti-MEM according to the table below.

		Small Scale Cell Culture Single Vector	Large Scale Cell Culture Single Vector
Positive control CTCF gRNA	**(dCas9_CTCF) vector**	1 µg	2 µg
	Opti-MEM	Up to 275 µl	Up to 550 µl
No gRNA negative control	**dCas9 vector**	1 µg	2 µg
	Opti-MEM	Up to 275 µl	Up to 550 µl

3. Add FuGENE HD Transfection reagent drop wise directly to the DNA/media mixture. Do not allow FuGENE to come directly in contact with the plastic from the tube. Mix the solution by pipetting up and down and incubate at room temperature for 30 minutes.
   Small scale cell culture: Add 6 µl FuGENE to each transfection reaction.
   Large scale cell culture: Add 12 µl FuGENE to each transfection reaction.
4. Add the entire DNA/media/FuGENE mixture drop wise to each cell culture plate. Incubate on a shaking platform at 100 rpm for 2 minutes to evenly distribute the transfection mixture.
5. Return plate to humidified incubator for 24 hours.
6. 24 hours post-transfection, passage cells.
   Small scale cell culture: Transfer cells in each well of a 6-well plate to a 10 cm dish.
   Large scale cell culture: Transfer cells from a 10 cm dish to a 15 cm dish.
7. Return plate to humidified incubator for 24 hours.
8. Transfected cells are now ready to be processed for chromatin fixation using the enChIP Kit (Catalog No. 53125). Alternatively, if duplicate reactions were performed cell lysates can be prepared using Active Motif's Nuclear Extraction Kit (Catalog No. 40010) for Western blot analysis of transfection efficiency using the AM-Tag polyclonal antibody (Catalog No. 61677) to recognize the tagged dCas9 protein.

Technical Services

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

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