A V I D I T Y, L.L.C.

Catalog# T5NRE p3A-T5-N-Avtg-RE Vector

This Avidity Atum AviTag ™ (AAA) Vector uses the T5 promoter (IPTG inducible) with an N-terminal AviTag, containing a rigid, extended linker. Please note that the vector is a linearized plasmid. The map of the vector is shown as circular for presentation purposes (see enclosed).

Cloning Vectors and Electra Cloning

The vector is supplied as 200ng linearized DNA (10 microliters, 20ng/microliter) or enough for 10 cloning reactions (see Cloning Protocol).

The Electra cloning kit is sold separately (50 reactions) as some customers may already have this kit. This kit is a vital part of the cloning step and comes with the Electra buffer (10x) and Electra Enzyme mix (20x). If you do not have the Electra cloning kit you may order it from us (Product # EKT-03, $200). **Please note**: the positive control from the Electra Enzyme kit will **not** work with the pA3 vectors.

Avidity p3A Positive Control (Please note that this Positive Control is not currently available).

Storage: Store at -**80ºC**.

Primer Design

Your ORF must **NOT** contain any SapI recognition sites, as the Electra[1] cloning process utilizes the type IIS enzyme SapI.

PCR Forward Primer for N- Terminal AviTag:
5′-TACACGTACTTAGTCGCTGAAGCTCTTCTTCTATG….(ORF)….

PCR Reverse Primer for N- Terminal AviTag:
5′-AGGTACGAACTCGATTGACGGCTCTTCTTTA….(ORF Reverse Complement)…

Appending the primer sequences (see above) to your forward and reverse PCR primers for cloning your target gene sequence. An additional 20 nucleotides of

ORF sequence from your target gene are the recommended length of ORF nucleotide sequence to be added to the primers.

Instructions for Use

PCR amplify the target gene using PCR primers as outlined above. Add the PCR product to a tube containing the p3A vector plus Electra Reagent mix. Incubate the reaction mix 5-20 minutes, then transform into Avidity BirA overexpressing strains (CVB100 or CVB101 for chemical transformation, EVB100 or EVB101 for electroporation).

From the Atum protocol for PCR products:

Component	Volume(µl)
PCR reaction	1 (20 ng DNA)
Avidity p3A Vector	1 (20 ng)
Electra Buffer Mix*	2
Electra Enzyme Mix*	1
Sterile ddH_2O	15
Total Volume	20

* *Electra Cloning Kit reagents*

1. Combine components as listed above in single 0.6 ml tube. Incubate at room temperature for 5-20 minutes.
2. Transform 2 µl of each reaction into competent cells.
3. Plate on LB + kanamycin (50 µg/ml)
4. Incubate plates overnight at 37°C. Pick transformants.

[1]Intellectual Property Statement Available online:
www.atum.bio/company/terms-and-conditions

If you have any questions, please do not hesitate to call Avidity.
Thank you for your order!