**Molecular Tech Fall 2017 Human Citrate Synthase Info**

**Citrate Synthase (CS) Human [>O75390.2:28-466]. N Term His Tag PLUS TEV**

 MDHHHHHHEN LYFQGASSTN LKDILADLIP KEQARIKTFR QQHGKTVVGQ

 ITVDMMYGGM RGMKGLVYET SVLDPDEGIR FRGFSIPECQ KLLPKAKGGE

 EPLPEGLFWL LVTGHIPTEE QVSWLSKEWA KRAALPSHVV TMLDNFPTNL

 HPMSQLSAAV TALNSESNFA RAYANGISRT KYWELIYEDS MDLIAKLPCV

 AAKIYRNLYR EGSGIGAIDS NLDWSHNFTN MLGYTDHQFT ELTRLYLTIH

 SDHEGGNVSA HTSHLVGSAL SDPYLSFAAA MNGLAGPLHG LANQEVLVWL

 TQLQKEVGKD VSDEKLRDYI WNTLNSGRVV PGYGHAVLRK TDPRYTCQRE

 FALKHLPNDP MFKLVAQLYK IVPNVLLEQG KAKNPWPNVD AHSGVLLQYY

 GMTEMNYYTV LFGVSRALGV LAQLIWSRAL GFPLERPKSM STEGLMKFVD

 SKSG

 TEV cleavage site is shown in red

 6xHis-tag is shown in blue

 N mutated to remove internal TEV cleavage site

cDNA Sequence of Citrate synthase [***Codon-optimized*** for expression in BL21 (DE3)].

ccATGGATCATCATCACCACCATCACGAGAACCTGTACTTCCAAGGTGCTAGCTCTACCAACCTGAAAGATATCCTGGCTGATCTGATTCCGAAGGAACAGGCACGCATCAAGACCTTCCGTCAGCAGCACGGTAAGACCGTAGTAGGTCAGATCACCGTGGACATGATGTACGGTGGCATGCGTGGTATGAAAGGCCTGGTATACGAAACCTCCGTGCTGGACCCAGACGAAGGCATTCGCTTCCGTGGTTTCTCTATCCCAGAATGTCAGAAACTGCTGCCGAAAGCGAAAGGTGGCGAAGAACCGCTGCCAGAAGGTCTGTTCTGGCTGCTGGTTACTGGTCACATCCCGACCGAGGAACAGGTGTCTTGGCTGTCTAAAGAATGGGCTAAACGTGCAGCACTGCCGTCTCATGTTGTGACCATGCTGGACAACTTCCCAACCAACCTGCATCCGATGTCTCAGCTGAGCGCTGCAGTTACTGCCCTGAACTCCGAATCTAACTTCGCACGTGCGTACGCCAACGGCATCTCTCGCACCAAGTATTGGGAACTGATCTACGAAGATTCTATGGACCTGATTGCTAAACTGCCGTGCGTAGCAGCGAAGATCTATCGTAACCTGTATCGTGAAGGTTCCGGTATTGGTGCAATCGACTCTAACCTGGACTGGAGCCATAACTTCACTAACATGCTGGGCTACACCGACCACCAGTTTACTGAACTGACTCGTCTGTATCTGACTATCCACAGCGATCACGAAGGTGGCAACGTGTCCGCACACACCTCTCACCTGGTAGGCAGCGCTCTGAGCGACCCGTACCTGTCCTTCGCAGCTGCTATGAACGGTCTGGCAGGTCCGCTGCACGGTCTGGCCAATCAGGAAGTTCTGGTATGGCTGACCCAGCTGCAGAAAGAAGTTGGTAAAGATGTTTCTGATGAGAAGCTGCGTGACTACATTTGGAACACTCTGAACAGCGGTCGTGTGGTTCCAGGTTACGGTCATGCTGTTCTGCGTAAGACCGATCCACGTTACACCTGCCAACGCGAGTTCGCTCTGAAACACCTGCCAAACGACCCGATGTTCAAACTGGTTGCGCAGCTGTACAAGATTGTGCCGAACGTTCTGCTGGAGCAAGGTAAAGCAAAGAACCCGTGGCCAAATGTTGACGCACACTCTGGTGTTCTGCTGCAATACTACGGCATGACCGAAATGAACTACTACACTGTGCTGTTCGGTGTGTCTCGTGCACTGGGTGTTCTGGCTCAGCTGATCTGGAGCCGTGCGCTGGGCTTTCCACTGGAACGTCCGAAATCCATGTCTACCGAGGGTCTGATGAAGTTCGTTGACAGCAAATCCGGTTAACTCGAG

Map of Citrate Synthase/AA28-466 in pET28a Vector



Synthetic gene was cloned into **NcoI/XhoI** digested **pET28a**.

Open reading frame orientation as illustrated. ***Not all unique restriction sites are shown in the map. Extra nucleotides or unique restriction sites may be found on both ends of your gene for subcloning purpose.***

**Seq:**

LOCUS GS61493-2 pET28a-hCitrate\_Synthase\_AA28-466 6597 bp ds-DNA circular SYN 25-Jun-2017

DEFINITION .

ACCESSION .

VERSION .

KEYWORDS GS61493-2 pET28a-hCitrate\_Synthase\_AA28-466

SOURCE synthetic DNA construct

 ORGANISM synthetic DNA construct

REFERENCE 1 (bases 1 to 6597)

 AUTHORS .

 TITLE Direct Submission

FEATURES Location/Qualifiers

 source 1..6597

 /organism="synthetic DNA construct"

 /mol\_type="other DNA"

 terminator 26..73

 /note="T7 terminator"

 /note="transcription terminator for bacteriophage T7 RNA

 polymerase"

 CDS complement(140..157)

 /codon\_start=1

 /product="6xHis affinity tag"

 /note="6xHis"

 /translation="HHHHHH"

 gene complement(158..1530)

 /note="hCitrate\_Synthase\_AA28-466"

 CDS complement(1505..1522)

 /codon\_start=1

 /product="6xHis affinity tag"

 /note="6xHis"

 /translation="HHHHHH"

 protein\_bind 1572..1596

 /bound\_moiety="lac repressor encoded by lacI"

 /note="lac operator"

 /note="The lac repressor binds to the lac operator to

 inhibit transcription in E. coli. This inhibition can be

 relieved by adding lactose or

 isopropyl-beta-D-thiogalactopyranoside (IPTG)."

 promoter complement(1597..1615)

 /note="T7 promoter"

 /note="promoter for bacteriophage T7 RNA polymerase"

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 /note="

 "

 CDS 2002..3084

 /codon\_start=1

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 /product="lac repressor"

 /note="lacI"

 /note="The lac repressor binds to the lac operator to

 inhibit transcription in E. coli. This inhibition can be

 relieved by adding lactose or

ORIGIN

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 61 ggggttatgc tagttattgc tcagcggtgg cagcagccaa ctcagcttcc tttcgggctt

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 181 caacgaactt catcagaccc tcggtagaca tggatttcgg acgttccagt ggaaagccca

 241 gcgcacggct ccagatcagc tgagccagaa cacccagtgc acgagacaca ccgaacagca

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