

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of active Diacylglycerol Kinase iota [1 - 1057]**

|                                                                              |                                      |
|------------------------------------------------------------------------------|--------------------------------------|
| <b><u>Enzyme description:-</u></b>                                           | DGK iota [1 - 1057]                  |
| <b><u>Clone number:-</u></b>                                                 | DU 34161                             |
| <b><u>Source:-</u></b>                                                       | Recombinant                          |
| <b><u>Expression system:-</u></b>                                            | Baculovirus expression vector system |
| <b><u>Tag:-</u></b>                                                          | N-terminal GST                       |
| <b><u>Purification method:-</u></b>                                          | Glutathione-Sepharose                |
| <b><u>Calculated molecular mass:-</u></b>                                    |                                      |
| Monoisotopic                                                                 | 144, 137.73 daltons                  |
| Average Mass                                                                 | 144, 045.64 daltons                  |
| [cysteines reduced, methionines have not been oxidised]                      |                                      |
| <b><u>Theoretical pI:-</u></b>                                               | 7.66                                 |
| <b><u>Purity:-</u></b>                                                       | >80 %                                |
| <b><u>Enzyme storage buffer:-</u></b>                                        |                                      |
| 50 mM HEPES pH 7.0, 150 mM NaCl, 20 % glycerol, 5 mM DTT                     |                                      |
| <b><u>Storage temperature:-</u></b>                                          | -70 °C                               |
| <b><u>Assay:</u></b>                                                         | ADP Glo                              |
| <b><u>Assay buffer:-</u></b>                                                 |                                      |
| 20 mM Tris-HCl, 67 mM KCl, 1 mM DTT, 0.05 mg/ml BSA, 10 mM MgCl <sub>2</sub> |                                      |
| <b><u>Substrate:-</u></b>                                                    |                                      |
| Diacylglycerol                                                               | Final concentration: 0.1 mM          |

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### **Clone Data Sheet**

#### **Diacylglycerol Kinase iota [1 - 1057]**

**Protein** DGK iota [1 - 1057]

**Clone number** DU 34161

**Species** Human

**Accession number** XP\_006716235

**Tags** N-terminal GST

**Baculovirus expressed protein**

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG  
LEFPNLPYYIDGDVKLTQSMATIRYIADKHNMLGGCPKERAETSMLEGA  
VLDIRYGVSRIAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH  
VTHPDFMLYDALDVLYMDPMCLDAFPKLVCFKKRIEAIPOIDKYLKSS  
KYIAWPLQGWQATFGGGDHPPKSDLEVLFGPLGSPGIPGSTRAAAAMD  
**AAGRGCHLLPLPAARGPARAPAAAAAASPPGPCSGAACAPSAAAGAG**  
**AMNPSSSAGEEKGATGGSSSSGSGAGSCCLGAEGGADPRGAGSAAAAGA**  
**AALDEPAAAGQKEKDEALEEKLRLNLTFRKQVSYRKAI SRAGLQHLAPAH**  
**PLSLPVANGPAKEPRATLDWSENAVNGEHLWLETNVSGDLCYLGEENCQ**  
**VRFAKSALRRKCAVCKIVVHTACIEQLEKINFRCKPTFREGGSRSPREN**  
**FVRHHWVHRRRQEGKCKQCGKGFQOKFSFHSKEIVAI SCWCKQAFHNK**  
**VTCFMLHHIEEPCSLGAHA AVIPPTWIIKVKKPQNSLKASNRKKKRTS**  
**FKRKASKRGMEQENKGRPFVIKPISSPLMKPLL VFVNP KSGGNQGTKVL**  
**QFMWYLNPRQVFDLSQEGPKDALELYRKVPNLRLACGGDGTVGWILS**  
**ILDELQLSPQPPVGVLP LGTGN DLARTLNWGGGYTDEPVSKILCQVEDG**  
**TVVQLDRWNLHVERNPDLPPEELEDGVCKLPLNVFN NYFSLGFD AHVTL**  
**EFHESREANPEKFNSRFRNKM FYAGAAFSDFLQRSSRDLSKHVKV VCDG**  
**TDLTPKIQELKFQCI VFLNIPRYCAGTMPWGNPGDHHDFEPQRHDDGYI**  
**EVIGFTMASLAALQVGGHGERLHQCREVMLLTYKSI PMQVDGEPCRLAP**  
**AMIRISLRNQANMVQKSKRRTSMPLLN DPQSVPDRLRIRVNKISLQDYE**  
**GFHYDKEKLREASIPLSILVVRGDCDLETCRMYIDRLQEDLQSVSSGSQ**  
**RVHYQDHETSFPRALSAQRLSPRWCFLDATSADR FYRIDRSQEHLHFVM**  
**EISQDEIFILDPDMVVSQ PAGTPPGMPDLVVEQASGISDWWNPALRKRM**  
**LSDSGLGMIAPYYEDSDLKDL SHSRVLQSPVSS EDHAILQAVIAGDLMK**  
**LIESYKNGGSLLIQGPDHCSLLHYAAKTGN GEIVKYILDHG PSELLDMA**  
**DSETGETALHKAACQRNRAVCQLLDAGASLRKTD SKGKTPQERAQQAG**  
**DPDLAAYLESRQNYKVIGHEDLETAV**

**Native sequence** Amino acids M1 – V1057 (end) of human DGK iota.  
Residue M244 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

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**Protease cleavage**      PreScission site (LEVLFQGP) residues 221 – 228

**Cloning sites**      *NotI* sites in pFastBAC GST

**Nucleotide  
sequence of insert**

gcggccgcgATGGACGCTGCTGGACGTGGTTGCCACCTGCTCCCCTGC  
CTGCTGCCAGAGGCCCCGCGCTGCTCCTGCTGCCGCTGCCGCTGCCGC  
TGCCTCGCCCCCTGGACCTTGCTCTGGAGCTGCTTGCGCTCCTTCCGCT  
GCCGCTGGTGCTGGAGCTATGAACCCATCCTCTAGCGCCGGCGAGGAAA  
AGGGCGCTACCGGTGGCTCATCGTCCTCTGGTTCTGGTGCTGGATCTTG  
CTGTCTGGGTGCTGAGGGAGGTGCTGATCCTCGTGGTGCTGGTTCCGCC  
GCTGCCGCTGGTGCCGCTGCCCTCGACGAGCCAGCTGCCGCTGGCCAGA  
AGGAGAAGGATGAAGCTTTGGAGGAAAAGTTGAGAAACCTGACTTTCCG  
TAAGCAGGTTTCTTACCGCAAGGCCATCAGCAGGGCTGGACTCCAACAC  
CTGGCTCCAGCTCACCTCTCTCCTTGCCAGTGGCTAACGGTCCAGCTA  
AGGAGCCGCGTGCCACACTGGACTGGTCTGAAAACGCTGTTAACGGCGA  
GCACCTGTGGCTCGAAACCAACGTGAGCGGAGATCTGTGCTACCTCGGC  
GAGGAAAACCTGTCAGGTCCGCTTCGCCAAGTCCGCTCTGCGTCGCAAGT  
GCGCCGTTTGTAAGATCGTGGTCCACACCGCTTGCATCGAGCAACTCGA  
AAAGATCAACTTCCGCTGCAAGCCTACTTTCCGTGAGGGCGGATCCAGG  
TCCCCAAGGGAAAACCTTCGTGAGGCACCACTGGGTCCACAGGAGACGTC  
AGGAGGGCAAGTGCAAGCAATGTGGAAAGGGTTTCCAGCAAAAGTTCTC  
ATTCCACTCGAAGGAGATCGTGGCCATCTCTTGCAGCTGGTGTAAGCAG  
GCTTTCCACAACAAGGTCACATGCTTCATGTTGCACCACATCGAGGAAC  
CATGCTCCCTGGGAGCTCACGCCGCTGTTATCGTGCCACCGACTTGGAT  
CATCAAGGTGAAGAAGCCCCAGAACAGCCTGAAGGCCTCAAACCGTAAG  
AAGAAGCGTACATCATTTCAAGAGAAAGGCTTCGAAGCGTGGCATGGAGC  
AAGAAAACAAGGGACGTCCCTTCGTGATCAAGCCATCAGCTCACCTTT  
GATGAAGCCATTGCTGGTCTTCGTAAACCCTAAGTCCGGTGGCAACCAG  
GGTACTAAGGTCCCTCAAATGTTTCATGTGGTACCTGAATCCACGGCAAG  
TCTTTGATCTTTCTCAGGAAGGGCCAAAAGATGCGCTTGAATTGTATAG  
GAAAGTACCAAATCTGCGAATTCTGGCCTGTGGTGGGGATGGAACGGTG  
GGCTGGATCCTTTCCATCCTGGATGAACTGCAGCTGAGCCCTCAGCCTC  
CTGTGGGGGTCTTCTCTGCGGACTGGGAATGACCTGGCTCGAACTCT  
CAACTGGGGAGGGGGCTACACTGATGAACCTGTTTCTAAGATCCTGTGT  
CAAGTGGAAGATGGGACAGTTGTACAGCTAGATCGCTGGAACCTCCATG  
TGGAAAGAAACCCCGACTTGCCCTCCAGAAGAACTTGAAGATGGCGTATG  
TAAGCTCCCTCTGAATGTTTTCAATAACTACTTCAGCCTTGGATTTGAT  
GCCCATGTACACTGGAGTTCCATGAATCCAGAGAAGCAAATCCAGAGA  
AATTCAACAGTCGTTTTTCGAAATAAAATGTTCTATGCAGGGGCAGCTTT  
TTCTGACTTCCTACAGAGAAGTTCTAGAGATCTATCCAAACATGTTAAA

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GTTGTTTGTGATGGAACAGATCTCACCCCAAAGATTCAGGAACTGAAGT  
TCCAGTGTATAGTATTTTTAAATATACCCAGATATTGTGCTGGCACAAT  
GCCCTGGGGAAACCCAGGTGATCACCATGATTTCTGAACCTCAGCGTCAT  
GATGATGGTTATATTGAAGTCATTGGATTTACCATGGCCTCTTTGGCAG  
CCCTGCAAGTTGGGGGCCATGGAGAGAGGCTACACCAGTGTGAGAAGT  
CATGCTTCTAACTTACAAATCCATCCCCATGCAAGTGGATGGGGAGCCC  
TGTAGGTTGGCCCCAGCTATGATTCGGATCTCCCTGAGGAATCAGGCCA  
ACATGGTACAGAAGAGCAAGAGGAGAACATCCATGCCTTTACTCAATGA  
TCCCCAGTCTGTCCCAGATCGTCTGAGGATCCGGGTGAACAAAATCAGT  
TTACAAGACTATGAAGGATTCCACTATGACAAGGAGAACTCCGAGAAG  
CTTCGATACCTCTGAGTATTCTAGTTGTGCGTGGAGACTGTGATTTGGA  
GACTTGCCGTATGTACATAGACCGCCTACAGGAGGACCTACAGTCAGTT  
TCTTCTGGCTCCCAGAGAGTTTCATTACCAGGACCATGAAACCTCCTTCC  
CCAGGGCTCTCTCAGCACAGAGGCTCTCTCCTCGGTGGTGCCTCCTAGA  
TGCAACTTCTGCTGATCGCTTTTATCGAATAGACAGATCTCAGGAACAT  
TTGCACTTTGTGATGGAGATTTCCCAAGATGAGATTTTATTCTGGACC  
CAGATATGGTGGTGTACACAGCCGGCGGGGACACCTCCGGGCATGCCTGA  
CCTGGTGGTGGAAACAAGCCTCGGGGATCTCAGACTGGTGGAATCCTGCC  
CTGCGGAAACGCATGCTGAGTGACAGTGGGCTGGGGATGATAGCTCCCT  
ATTATGAGGACTCAGATCTGAAAGATCTCAGCCACTCCCGCGTGCTACA  
GTCACCAGTCTCTTCAGAGGATCATGCAATTTTGCAGGCAGTAATAGCT  
GGTGATCTTATGAAGCTAATAGAAAGCTATAAAAATGGAGGCAGTCTGC  
TAATTCAGGGACCAGACCACTGTTCACTCCTTCACTACGCAGCTAAAAC  
CGGCAACGGGGAGATTGTGAAATATATCCTTGACCACGGACCTTCCGAG  
TTATTGGATATGGCAGACAGTGAAACGGGTGAGACTGCACTGCACAAGG  
CTGCCTGCCAGCGGAACCGGGCTGTGTGCCAGCTTCTGGTGGATGCAGG  
AGCATCTCTGAGAAAGACGGACTCCAAGGGTAAGACACCTCAAGAAAGA  
GCACAGCAGGCTGGGGACCCAGACTTGGCTGCTTACCTAGAAAGCCGTC  
AGAACTATAAGGTCATTGGCCATGAGGACCTGGAAACTGCTGTTTGAgc  
ggccgc