Certificate of Analysis

Order Number: 520141-4

Protein Name: Thymidylate kinase

Shipping Conditions: Dry Ice

Lot Number: 520141S04/P20011412

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Certificate of Analysis

Order Number:	520141-4
Protein Name:	Thymidylate kinase
Shipping Conditions:	Dry Ice
Lot Number:	520141S04/P20011412

Expression System:	*E.coli*
Purification:	Protein was obtained from inclusion bodies

Package:	5.00 mg. 1.00 ml/tube, 5 tubes
Concentration:	1 mg/ml, as determined by Bradford protein assay with BSA as a standard
Purity:	About 85% as estimated by densitometric analysis of the Coomassie Blue-stained SDS-PAGE gel
Sterility:	Sterilized via a 0.22 μm filter and packaged aseptically
Storage and Handling:	Store at -80°C. Aliquots should be stored at the same temperature after first use to avoid multiple freeze-thaws
Storage Buffer:	50 mM Tris-HCl, 150 mM NaCl, 10% Glycerol, 500 mM L-Arginine, pH 8.0

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SDS-PAGE Analysis:

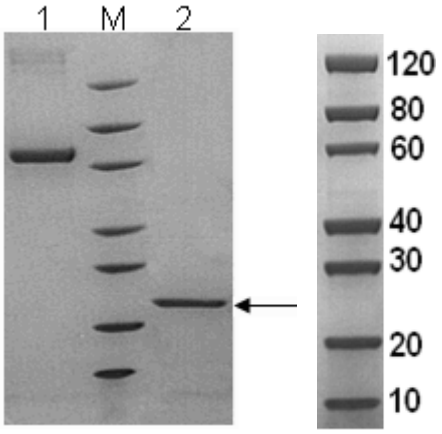
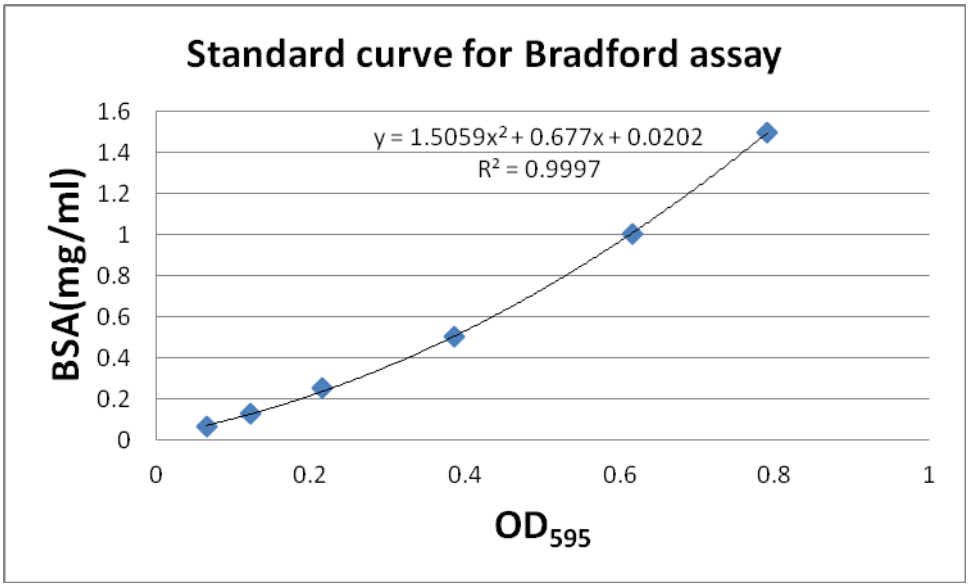


Fig.1 SDS-PAGE analysis
Lane 1: BSA (2.00 μg)
Lane 2: Thymidylate kinase (2.00 μg)

The SDS-PAGE was run on 4%~20% gradient gel, followed by Coomassie Blue staining
Protein Marker M: GenScript, Cat. No. M00516

Quantification--BSA Standard Curve:



Sample	Abs at 595 nm(diluted 2 times)	Protein (mg/ml)
Thymidylate kinase	0.383	1 mg/ml

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Packing List of 520141-4

Protein (Store at -80°C)

Protein name: Thymidylate kinase
tag free
Purity: >85%
Concentration: 1 mg/ml
Total: 5.00 mg. 1.00 ml/tube, 5 tubes

Certified by: **Date:** ***12/29/2014***

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Appendix

DNA Sequence (Sequencing Confirmed):

```
   1  ATGCTGATTG  CTATTGAAGG  TGTTGATGGT  GCTGGTAAAC  GTACGCTGGT  TGAAAAACTG
  61  TCTGGTGCTT  TCCGTGCGGC  GGGTCGTAGC  GTGGCTACCC  TGGCGTTTCC  GCGCTATGGC
 121  CAGAGTGTTG  CGGCCGATAT  TGCAGCTGAA  GCCCTGCATG  GCGAACACGG  TGACCTGGCA
 181  AGCTCTGTCT  ATGCTATGGC  AACCCTGTTC  GCACTGGATC  GTGCAGGTGC  AGTGCATACG
 241  ATTCAGGGCC  TGTGCCGCGG  TTACGATGTG  GTTATCCTGG  ACCGTTATGT  TGCCAGTAAC
 301  GCGGCCTACT  CCGCAGCTCG  TCTGCACGAA  AATGCAGCCG  GTAAAGCAGC  TGCATGGGTC
 361  CAGCGCATCG  AATTTGCGCG  TCTGGGTCTG  CCGAAACCGG  ATTGGCAAGT  TCTGCTGGCC
 421  GTCTCAGCAG  AACTGGCTGG  TGAACGTTCG  CGTGGTCGTG  CACAGCGTGA  CCCGGGTCGT
 481  GCTCGTGATA  ACTATGAACG  CGACGCGGAA  CTGCAGCAAC  GTACGGGTGC  AGTGTACGCA
 541  GAACTGGCAG  CACAAGGTTG  GGGCGGTCGT  TGGCTGGTCG  TGGGTGCTGA  TGTGGACCCG
 601  GGCCGTCTGG  CTGCAACCCT  GGCTCCGCCG  GATGTGCCGT  CG
```


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Derived Protein Sequence:

Theoretical Isoelectric Point	Theoretical Molecular Weight
7.66	22635.0 Da

```
  1  MLIAIEGVDG  AGKRTLVEKL  SGAFRAAGRS  VATLAFPRYG  QSVAADIAAE  ALHGEHGDLA
 61  SSVYAMATLF  ALDRAGAVHT  IQGLCRGYDV  VILDRYVASN  AAYSAARLHE  NAAGKAAAWV
121  QRIEFARLGL  PKPDWQVLLA  VSAELAGERS  RGRAQRDPGR  ARDNYERDAE  LQQRTGAVYA
181  ELAAQGWGGR  WLVVGADVDP  GRLAATLAPP  DVPS
```


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1. Gene Synthesis and Subcloning:

After customer's approval, target DNA sequence 520141-4(Thymidylate kinase) was designed, synthesized with related tags for each construct to facilitate the purification. The complete sequence was subcloned into target vectors for *E. coli* expression.

2. Expression Evaluation:

E. coli BL21 (DE3) was transformed with recombinant plasmids. A single colony was inoculated into medium containing ampicillin or kanamycin; cultures were incubated in 37 °C at 200 rpm. IPTG was introduced for induction. SDS-PAGE was used to monitor the expression.

3. Purification and Analysis:

Cells were harvested by centrifugation. Cell pellets were lysed by sonication, and then precipitate after centrifugation was dissolved using urea. Fractions were pooled and refolded followed by 0.22 μm filter sterilization. Proteins were analyzed by MALDI-TOF and SDS-PAGE by using standard protocols for molecular weight and purity measurements. The concentration was determined by Bradford protein assay with BSA as a standard.

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