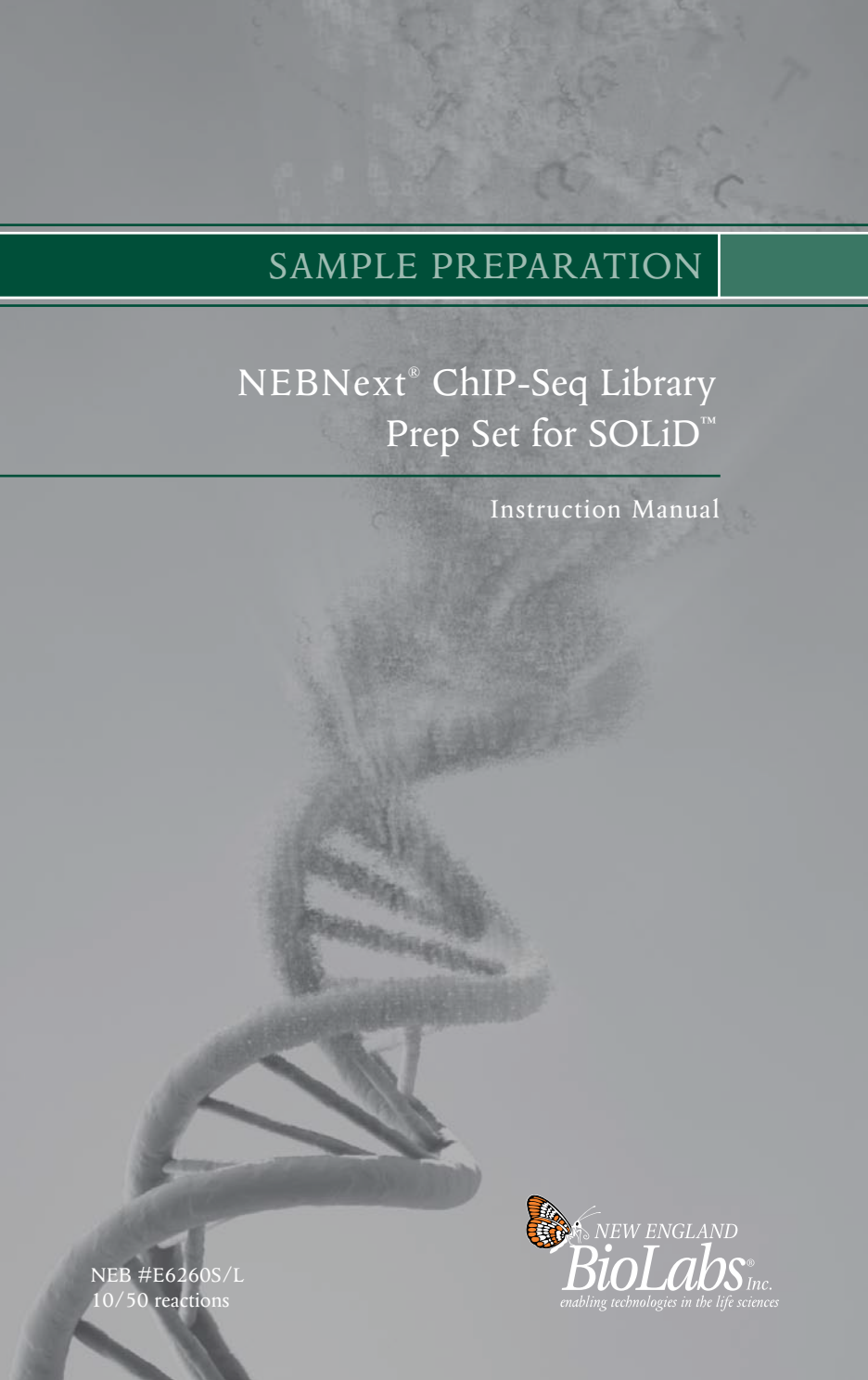
SAMPLE PREPARATION

NEBNext® ChIP-Seq Library Prep Set for SOLiD™

Instruction Manual



NEB #E6260S/L
10/50 reactions

NEW ENGLAND BioLabs® Inc.
enabling technologies in the life sciences

NEBNext ChIP-Seq Library Prep Set for SOLiD

Table of Contents:

The Reagent Set Includes:

The volumes provided are sufficient for preparation of up to 10 reactions (NEB #E6260S) and 50 reactions (NEB #E6260L). (All reagents should be stored at –20°C):

NEBNext End Repair Enzyme Mix RR

NEBNext End Repair Reaction Buffer (10X)

Quick T4 DNA Ligase RR

NEBNext Quick Ligation Reaction Buffer (5X)

LongAmp *Taq* 2X Master Mix RR

Cloned at NEBiolabs | RR Recombinant Enzyme | NEB2 Optimum Buffer | BSA Requires BSA | Yes Heat Inactivation

Applications:

The NEBNext ChIP-Seq Library Prep Set for SOLiD contains enzymes and buffers in convenient master mix formulations that are ideally suited for sample preparation for next-generation sequencing of Chromatin Immunoprecipitated DNA. Each of these components must pass rigorous quality control standards and are lot controlled, both individually and as a set of reagents.

Lot Control: The lots provided in the NEBNext ChIP-Seq Library Prep Set for SOLiD are managed separately and are qualified by additional functional validation. Individual reagents undergo standard enzyme activity and quality control assays, and also meet stringent criteria in the additional quality controls listed on each individual component page.

Functional Validation: Each set of reagents is functionally validated together through construction and sequencing of a ChIP DNA library on a SOLiD System (Life Technologies, Inc.).

For larger volume requirements, customized and bulk packaging is available by purchasing through the OEM/Bulks department at NEB. Please contact OEM@neb.com for further information.

Protocols:

Starting Material: 10 ng of chromatin-immunoprecipitated (ChIP) qPCR verified or control DNA in ≤ 85 µl of water or elution buffer

NEBNext End Repair Module Protocol

1. Mix the following components in a sterile microfuge tube:

ChIP DNA	1–85 µl
NEBNext End Repair Reaction Buffer (10X)	10 µl
NEBNext End Repair Enzyme Mix	5 µl
Sterile H_2O	variable
Total volume	100 µl

2. Incubate for 30 minutes at room temperature.
3. Purify the end repaired DNA using Agencourt AMPure® XP beads (Beckman Coulter, Inc.).

Agencourt AMPure XP Bead Clean-up and Size Selection of End Repaired DNA

1. Warm the AMPure beads to room temperature and mix thoroughly before use.
2. Prepare 3.6 mls of 70% ethanol for each sample. The 70% ethanol solution should be prepared fresh.
3. Add 70 µl of AMPure beads to each sample, mix thoroughly and rotate for 10 minutes at room temperature.
4. Place samples on a magnetic separator. When the beads have collected to the wall of the tube and the solution is clear, **transfer** the liquid to a new tube. Be careful not to disturb the beads. The liquid contains the end repaired DNA.
5. Add 110 µl of AMPure beads to each sample, mix and rotate for 10 minutes at room temperature.
6. Place the samples on a magnetic separator, when the beads have collected to the wall of the tube and the solution is clear, remove and discard the liquid. The end repaired DNA is now bound to the beads.
7. Add 300 µl of 70% ethanol. Wash the beads by turning the tube 180° and allowing the beads to re-collect on the side of the tube. Turn the tube 6 times.
8. Remove and discard the ethanol.
9. Repeat steps 7 and 8 two more times.
10. Remove the tubes from the magnetic separator, quick spin the beads, place back on the magnet and remove any remaining liquid. The quick spin will aid in drying the beads.
11. Keeping the tubes on the magnet and the caps open, dry the beads at room temperature for 20–30 minutes. Cracks will be observed in the bead pellet when drying is complete.
12. Add 30 µl of water to the dried beads and vortex to mix thoroughly.
13. `Place the samples on a magnetic separator, when the beads have collected to the wall of the tube and the solution is clear, transfer the liquid to a fresh tube. The liquid contains your purified library.

Adaptor Ligation of End Repaired DNA

1. Mix the following components in a sterile microfuge tube:

End Repaired, Blunt DNA	30 µl
NEBNext Quick Ligation Reaction Buffer (5X)	20 µl
*P1 Adaptor (2.5 pmol / µl)	1 µl
*P2 Adaptor (2.5 pmol / µl)	1 µl
Quick T4 DNA Ligase	1 µl
Sterile H_2O	47 µl
Total volume	100 µl

**Adaptors are not included, use adaptors appropriate to specific application.*

2. Incubate at room temperature for 10 minutes.

Purification of Adaptor Ligated DNA

1. Warm AMPure beads to room temperature and mix thoroughly before use.
2. Add 180 µl of AMPure beads to each sample, mix and rotate for 10 minutes at room temperature.
3. Place the samples on a magnetic separator, when the beads have collected to the wall of the tube and the solution is clear, remove and discard the liquid.
4. Add 300 µl of freshly prepared 70% ethanol to the beads. Wash by turning the tube 180° and allowing the beads to re-collect on the side of the tube. Turn the tube 6 times.
5. Remove and discard the ethanol, be careful not to disturb the beads.
6. Repeat steps 4 and 5 two more times.
7. Remove the tubes from the magnetic separator, quick spin the beads, place them back on the magnet and remove any remaining liquid. The quick spin will aid in drying the beads.
8. Keeping the tubes on the magnet and the caps open, dry the beads at room temperature for 20–30 minutes. Cracks will be observed in the bead pellet when drying is complete.
9. Add 30 µl of water to the dried beads and vortex to mix thoroughly.
10. Place the samples on a magnetic separator, when the beads have collected to the wall of the tube and the solution is clear, transfer the liquid to a fresh tube. The liquid contains your purified adaptor ligated library.

Nick Translation and Amplification of Adaptor Ligated DNA

1. Mix the following components in a sterile microfuge tube:

Adaptor ligated DNA	30 µl
Primer 1 (50 µM stock)	5 µl
Primer 2 (50 µM stock)	5 µl
LongAmp *Taq* 2X Master Mix	125 µl
Sterile H_2O	85 µl
Total volume	250 µl

2. Aliquot 125 µl into two PCR tubes.
3. PCR cycling conditions:

CYCLE STEP	TEMP	TIME	CYCLES
Nick Translation	72°C	20 min	1
Initial Denaturation	95°C	5 min	1
Denaturation	95°C	15 sec	10–15*
Annealing	62°C	15 sec	
Extension	70°C	1 min	
Final Extension	70°C	5 min	1
Hold	4°C	∞	1

**Avoid over-amplification to optimize the number of unique molecules.*

Purification of Amplified DNA

1. Warm AMPure beads to room temperature and mix thoroughly before use.
2. Add 450 µl of AMPure beads to each sample, mix and rotate for 10 minutes at room temperature.
3. Place the samples on a magnetic separator, when the beads have collected to the wall of the tube and the solution is clear, remove and discard the liquid.
4. Add 300 µl of freshly prepared 70% ethanol to the beads. If the beads are not submerged in the ethanol, quick spin the beads and place back on the magnet. Wash by turning the tube 180° and allowing the beads to re-collect on the side of the tube. Turn the tube 6 times.
5. Remove and discard the ethanol, be careful not to disturb the beads.
6. Repeat steps 4 and 5 two more times.
7. Remove the tubes from the magnetic separator, quick spin the beads, place them back on the magnet and remove any remaining liquid. The quick spin will aid in drying the beads.
8. Keeping the tubes on the magnet and the caps open, dry the beads at room temperature for 20–30 minutes. Cracks will be observed in the bead pellet when drying is complete.
9. Add 30 µl of water to the dried beads and vortex to mix thoroughly.
10. Place the samples on a magnetic separator, when the beads have collected to the wall of the tube and the solution is clear, transfer the liquid to a fresh tube. The liquid contains your amplified library.

Perform a Second Purification of the Amplified DNA

1. Warm AMPure beads to room temperature and mix thoroughly before use.
2. Add 54 µl of AMPure beads to each sample, mix and rotate for 10 minutes at room temperature.
3. Place the samples on a magnetic separator, when the beads have collected to the wall of the tube and the solution is clear, remove and discard the liquid.
4. Add 300 µl of freshly prepared 70% ethanol to the beads. If the beads are not submerged in the ethanol, quick spin the beads and place back on the magnet. Wash by turning the tube 180° and allowing the beads to re-collect on the side of the tube. Turn the tube 6 times.
5. Remove and discard the ethanol, be careful not to disturb the beads.
6. Repeat steps 4 and 5 two more times.
7. Remove the tubes from the magnetic separator, quick spin the beads, place them back on the magnet and remove any remaining liquid. The quick spin will aid in drying the beads.
8. Keeping the tubes on the magnet and the caps open, dry the beads at room temperature for 20–30 minutes. Cracks will be observed in the bead pellet when drying is complete.
9. Add 30 µl of water to the dried beads and vortex to mix thoroughly.
10. Place the samples on a magnetic separator, when the beads have collected to the wall of the tube and the solution is clear, transfer the liquid to a fresh tube. The liquid contains your amplified library.

NEBNext End Repair Enzyme Mix

#E6261A: 0.05 ml
#E6261AA: 0.25 ml

Store at –20°C

Description: NEBNext End Repair Enzyme Mix is optimized to convert fragmented to repaired DNA having 5´-phosphorylated, blunt ends.

NEBNext End Repair Enzyme Mix:
10,000 units/ml T4 Polynucleotide Kinase
3,000 units/ml T4 DNA Polymerase

Storage Conditions:
10 mM Tris-HCl
100 mM KCl
1 mM DTT
0.1 mM EDTA
50% Glycerol
0.1% Triton X-100
pH 7.4 @ 25°C

Quality Control Assays

SDS-PAGE Purity: SDS-PAGE analyses of each individual enzyme indicates > 95% enzyme purity.

Endonuclease Activity: Incubation of a minimum of 10 µl of this enzyme mix with 1 µg of ϕX174 RF I DNA in assay buffer for 4 hours at 37°C in 50 µl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

Phosphatase Activity: Incubation of a minimum of 10 µl of this enzyme mix in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

Functional Activity (Nucleotide Incorporation): 0.2 µl of this enzyme mix incorporates 10 nmol of dNTP into acid-precipitable material in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X T4 DNA Polymerase Reaction Buffer with 33 µM dNTPs including [^{3}H]-dTTP, 70 µg/ml denatured herring sperm DNA and 50 µg/ml BSA.

Functional Activity (Nucleotide Incorporation and Phosphorylation): 5 µl of this enzyme mix repairs and phosphorylates the ends of > 95% of 10 µg of DNA fragments containing both 3´ and 5´ overhangs within 30 minutes at 20°C in 1X End Repair Buffer, as determined by capillary electrophoresis.

Lot Controlled

NEBNext End Repair Reaction Buffer

#E6262A: 0.1 ml **Concentration: 10X**
#E6262AA: 0.5 ml

Store at –20°C

1X NEBNext End Repair Reaction Buffer:
50 mM Tris-HCl
10 mM $MgCl_2$
10 mM DTT
1 mM ATP
0.4 mM dATP
0.4 mM dCTP
0.4 mM dGTP
0.4 mM dTTP
pH 7.5 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing this reaction buffer at 1X concentration and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing this reaction buffer at 1X concentration and 1 µg T3 DNA incubated for 16 hours at 37°C also results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

Endonuclease Activity: Incubation of this reaction buffer at a 1X concentration with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

RNase Activity: Incubation of this reaction buffer at 1X concentration with 40 ng of a FAM-labeled RNA transcript for 16 hours at 37°C results in no detectable RNase activity as determined by polyacrylamide gel electrophoresis.

Phosphatase Activity: Incubation of this reaction buffer at a 1X concentration in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

Lot Controlled

Quick T4 DNA Ligase

#E6263A: 0.015 ml
#E6263AA: 0.050 ml

RR Yes

Store at –20°C

Source: Purified from *E. coli* C600 pcl857 pPLc28 lig8 (2).

Quality Control Assays

SDS-PAGE Purity: SDS-PAGE analysis of this enzyme indicates > 95% enzyme purity.

16-Hour Incubation: 50 µl reactions containing a minimum of 2,000 units of this enzyme and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing a minimum of 2,000 units of this enzyme and 1 µg T3 DNA incubated for 16 hours at 37°C also results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

Endonuclease Activity: Incubation of a minimum of 3,200 units of this enzyme with 1 µg of ϕX174 RF I DNA in assay buffer for 4 hours at 37°C in 50 µl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

Phosphatase Activity: Incubation of a minimum of 20,000 units of this enzyme in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

RNase Activity: Incubation of a minimum of 2,000 units of this enzyme with 40 ng of a FAM-labeled RNA transcript for 16 hours at 37°C results in no detectable RNase activity as determined by polyacrylamide gel electrophoresis.

Exonuclease Activity: Incubation of a minimum of 3,200 units of this enzyme with 1 µg sonicated [^{3}H] DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer releases < 0.1% radioactivity.

Functional Activity (Blunt End Ligation): 50 µl reactions containing a 0.5 µl T4 DNA Ligase, 18 µg HindIII digested ϕX174 and 1X T4 DNA Ligase Buffer incubated at 16°C for 7.5 min results in > 95% of fragments ligated as determined by agarose gel electrophoresis.

Functional Activity (Cohesive End Ligation): 20 µl reactions containing 0.5 µl T4 DNA Ligase, 12 µg HindIII digested lambda DNA and 1X T4 DNA Ligase Buffer incubated at 37°C overnight results in > 95% of fragments ligated as determined by agarose gel electrophoresis. Redigestion of the ligated products, 50 µl reactions containing 6 µg of the ligated fragments, 40 units HindIII, and 1X NEBuffer 2 incubated at 37°C for 2 hours, results in no detectable undigested fragments as determined by agarose gel electrophoresis.

Functional Activity (Adaptor Ligation): 50 µl reactions containing 0.125 µl T4 DNA Ligase, 8 nmol 12 bp adaptor, and 1X T4 DNA Ligase Buffer incubated at 16°C overnight results in no detectable unligated adaptor as determined by agarose gel electrophoresis.

Functional Activity (Transformation): After a five-minute ligation of linearized, dephosphorylated LITMUS™ 28 (containing either blunt [EcoRV] or cohesive [HindIII] ends) and a mixture of compatible insert fragments, transformation into chemically competent *E. coli* DH-5 alpha cells yields a minimum of 1 x 10^6 recombinant transformants per µg plasmid DNA.

Lot Controlled

References:

1. Engler, M. J. and Richardson, C. C. (1982). In P. D. Boyer (Ed.), *The Enzymes* Vol. 5, (p. 3). San Diego: Academic Press.
2. Remaut, E., Tsao, H. and Fiers, W. (1983) *Gene,* 22, 103–113.

NEBNext Quick Ligation Reaction Buffer

#E6264A: 0.2 ml **Concentration: 5X**
#E6264AA: 1.0 ml

Store at –20°C

1X NEBNext Quick Ligation Reaction Buffer:
66 mM Tris-HCl
10 mM $MgCl_2$
1 mM dithiothreitol
1 mM ATP
6% Polyethylene glycol (PEG 6000)
pH 7.6 @ 25°C

Quality Control Assays

16-Hour Incubation: 50 µl reactions containing this reaction buffer at 1X concentration and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis. 50 µl reactions containing this reaction buffer at 1X concentration and 1 µg T3 DNA incubated for 16 hours at 37°C also results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

Endonuclease Activity: Incubation of this reaction buffer at a 1X concentration with 1 µg of ϕX174 RF I DNA for 4 hours at 37°C in 50 µl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

RNase Activity: Incubation of this reaction buffer at 1X concentration with 40 ng of a FAM-labeled RNA transcript for 16 hours at 37°C results in no detectable RNase activity as determined by polyacrylamide gel electrophoresis.

Phosphatase Activity: Incubation of this reaction buffer at a 1X concentration in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

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LongAmp *Taq* 2X Master Mix

E6065A: 1.25 ml **Concentration: 2X**
E6065AA: 6.25 ml

Store at –20°C

1X LongAmp *Taq* Master Mix:
60 mM Tris-SO_4
2 mM $MgSO_4$
0.3 mM dNTPs
125 units/ml LongAmp *Taq* DNA Polymerase
20 mM ammonium sulfate
5% glycerol
0.06% NP-40
0.05% Tween-20
pH 9.0 @ 25°C

Quality Control Assays

Long Amplicon PCR: The master mix is tested for the ability to amplify a 30 kb amplicon from lambda DNA and a 30 kb fragment from human genomic DNA.

SDS-PAGE Purity: SDS-PAGE analyses of each individual enzyme indicates > 95% enzyme purity.

16-Hour Incubation: 50 µl reactions containing a minimum of 5 units of this enzyme and 1 µg of HindIII digested Lambda DNA incubated for 16 hours at 37°C results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.50 µl reactions containing a minimum of 5 units of this enzyme and 1 µg T3 DNA incubated for 16 hours at 37°C also results in no detectable non-specific nuclease degradation as determined by agarose gel electrophoresis.

Endonuclease Activity: Incubation of a minimum of 10 µl of this enzyme mix with 1 µg of ϕX174 RF I DNA in assay buffer for 4 hours at 37°C in 50 µl reactions results in less than 10% conversion to RF II as determined by agarose gel electrophoresis.

Phosphatase Activity: Incubation of a minimum of 10 µl of this enzyme mix in protein phosphatase assay buffer (1 M diethanolamine @ pH 9.8 and 0.5 mM $MgCl_2$) containing 2.5 mM *p*-nitrophenyl phosphate at 37°C for 4 hours yields no detectable *p*-nitrophenylene anion as determined by spectrophotometric analysis at 405 nm.

HPLC: dNTP purity is determined by HPLC to be > 99%.

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