# RecA

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

M0249S 027160118011

# M0249S

RR 65°

| | | |
|---|---|---|
| **200 µg** | **2 mg/ml** | **Lot: 0271601** |
| **RECOMBINANT** | **Store at –20°C** | **Exp: 1/18** |

**Description:** *E. coli* RecA is necessary for genetic recombination, reactions involving DNA repair and UV-induced mutagenesis. RecA promotes the autodigestion of the lexA repressor, umuD protein and lambda repressor. Cleavage of LexA derepresses more than 20 genes (1). *In vitro* studies indicate that in the presence of ATP, RecA promotes the strand exchange of single-strand DNA fragments with homologous duplex DNA. The reaction has three distinct steps: (i) RecA polymerizes on the single-strand DNA, (ii) the nucleoprotein filament binds the duplex DNA and searches for a homologous region, (iii) the strands are exchanged (2).

**Source:** An *E. coli* strain ER2502 that carries an overexpressed RecA gene from *E. coli*.

**Applications:**

- Visualization of DNA structures with electron microscopy (3)
- D-loop mutagenesis (4)
- Screening libraries using RecA-coated probes (5,6)
- Cleavage of DNA at any single predetermined site (7,8,9)
- RecA mediated affinity capture for full length cDNA cloning (10,11)

Supplied in: 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM dithiothreitol and 50% glycerol.

**Reagents Supplied with Enzyme:**
10X RecA Reaction Buffer.

**Note:** ATPγS which is required for triple helix formation is not supplied.

**Reaction Conditions:**
1X RecA Reaction Buffer. Incubate at 37°C.

**1X RecA Reaction Buffer:**
70 mM Tris-HCl
10 mM $MgCl_2$
5 mM dithiothreitol
pH 7.6 @ 25°C

**Molecular Weight:** 37,973 daltons.

**Quality Assurance:** Each lot is tested for its ability to form a stable triple helix and is visually determined to be > 99% pure on an SDS-polyacrylamide gel.

## Quality Control Assays

**Exonuclease Activity (Radioactivity Release):** A 50 µl reaction in RecA Reaction Buffer containing 1 µg of a mixture of single and double-stranded [$^3$H] *E. coli* DNA and 10 µg of RecA incubated for 4 hours at 37°C releases < 0.1% of the total radioactivity.

**Endonuclease Activity (Nicking):** A 50 µl reaction in RecA Reaction Buffer containing 1 µg of supercoiled ϕX174 RF I DNA and 10 µg of RecA incubated for 4 hours at 37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

**Non-Specific DNase Activity (16 hour):** A 50 µl reaction in RecA Reaction Buffer containing 1 µg of λ DNA and 10 µg of RecA incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**RNase Activity (Extended Digestion):** A 50 µl reaction in RecA Reaction Buffer containing 40 ng of labeled RNA and 10 µg of RecA is incubated at 37°C. After incubation for 4 hours, > 90% of the substrate RNA remains intact as determined by agarose gel.

**Molecular Weight Determination (Mass Spectrometry):** The molecular weight of RecA is between 37,963and 37,983 as determined by mass spectrometry analysis.

*(see other side)*

CERTIFICATE OF ANALYSIS

RecA

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

M0249S 027160118011

M0249S

RR 65°

200 µg | 2 mg/ml | Lot: 0271601
RECOMBINANT | Store at –20°C | Exp: 1/18

**Description:** *E. coli* RecA is necessary for genetic recombination, reactions involving DNA repair and UV-induced mutagenesis. RecA promotes the autodigestion of the lexA repressor, umuD protein and lambda repressor. Cleavage of LexA derepresses more than 20 genes (1). *In vitro* studies indicate that in the presence of ATP, RecA promotes the strand exchange of single-strand DNA fragments with homologous duplex DNA. The reaction has three distinct steps: (i) RecA polymerizes on the single-strand DNA, (ii) the nucleoprotein filament binds the duplex DNA and searches for a homologous region, (iii) the strands are exchanged (2).

**Source:** An *E. coli* strain ER2502 that carries an overexpressed RecA gene from *E. coli*.

**Applications:**

- Visualization of DNA structures with electron microscopy (3)
- D-loop mutagenesis (4)
- Screening libraries using RecA-coated probes (5,6)
- Cleavage of DNA at any single predetermined site (7,8,9)
- RecA mediated affinity capture for full length cDNA cloning (10,11)

Supplied in: 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM dithiothreitol and 50% glycerol.

**Reagents Supplied with Enzyme:**
10X RecA Reaction Buffer.

**Note:** ATPγS which is required for triple helix formation is not supplied.

**Reaction Conditions:**
1X RecA Reaction Buffer. Incubate at 37°C.

**1X RecA Reaction Buffer:**
70 mM Tris-HCl
10 mM $MgCl_2$
5 mM dithiothreitol
pH 7.6 @ 25°C

**Molecular Weight:** 37,973 daltons.

**Quality Assurance:** Each lot is tested for its ability to form a stable triple helix and is visually determined to be > 99% pure on an SDS-polyacrylamide gel.

## Quality Control Assays

**Exonuclease Activity (Radioactivity Release):** A 50 µl reaction in RecA Reaction Buffer containing 1 µg of a mixture of single and double-stranded [$^3$H] *E. coli* DNA and 10 µg of RecA incubated for 4 hours at 37°C releases < 0.1% of the total radioactivity.

**Endonuclease Activity (Nicking):** A 50 µl reaction in RecA Reaction Buffer containing 1 µg of supercoiled ϕX174 RF I DNA and 10 µg of RecA incubated for 4 hours at 37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

**Non-Specific DNase Activity (16 hour):** A 50 µl reaction in RecA Reaction Buffer containing 1 µg of λ DNA and 10 µg of RecA incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**RNase Activity (Extended Digestion):** A 50 µl reaction in RecA Reaction Buffer containing 40 ng of labeled RNA and 10 µg of RecA is incubated at 37°C. After incubation for 4 hours, > 90% of the substrate RNA remains intact as determined by agarose gel.

**Molecular Weight Determination (Mass Spectrometry):** The molecular weight of RecA is between 37,963and 37,983 as determined by mass spectrometry analysis.

*(see other side)*

CERTIFICATE OF ANALYSIS

**Protein Concentration Determination:** The concentration of RecA is between 1.9 and 2.1 mg/ml as determined by UV absorption at 280 nm by the Pace method using the extinction coefficient of 21,555 and molecular weight of 37,973 daltons (12).

**RecA Functional Assay (Triple Helix Formation):** The plasmid pUC19 contains 5 HpyCH4 IV sites. A 60 mer was designed with complementarity to the region centered around the HpyCH4 IV site at position 374. A reaction containing 1 µg pUC19, 0.18 µg 60 mer, 0.3 mM ATP γ-S, 4 µg RecA, in 40 µl 1X RecA Reaction Buffer was incubated at 37°C for 10 minutes to form a stable triple helix. The unprotected sites were methylated using 8 units of Sss I supplemented with 160 µM SAM for 10 minutes at 37°C. The reaction was stopped and the triple helix was disrupted by incubation at 65°C for 15 minutes. The reaction was cooled and 10 units of HpyCH4 IV were added followed by digestion at 37°C for 20 minutes. > 90% of the product is single cut pUC19.

**Heat Inactivation:** 65°C for 20 minutes.



**References**:


1. West, S.C. (1992) *Ann. Rev. Biochem.* 61, 603–640.
2. Radding, C.M. (1991) *J. Biol. Chem.* 266, 5355–5358.
3. Wasserman, S.A. and Cozzarelli, N.R. (1985) *Proc. Natl. Acad. Sci. USA* 82, 1079–1083.
4. Shortle, D. et al. (1980) *Proc. Natl. Acad. Sci. USA* 77, 5375–5379.
5. Honigberg, S.M. et al. (1986) *Proc. Natl. Acad. Sci. USA* 83, 9586–9590.
6. Rigas, B. et al. (1986) *Proc. Natl. Acad. Sci. USA* 83, 9591–9595.
7. Ferrin, L.J. and Camerini-Otero, R.D. (1991) *Science* 254, 1494–1497.
8. Koob, M. et al. (1992) *Nucleic Acids Res.* 20, 5831–5836.
9. Koob, M. (1992). In R. Wu (Ed.), *Methods in Enzymology* Vol. 216, (pp. 321–329). San Diego: Academic Press.
10. Zhumabayeva, B. et al. (1990) *Biotechniques* 27, 834–845.
11. Zhumabayeva, B. et al. (2001) *Biotechniques* 30, 512–520.
12. Pace, C.N. et al. (1995) Protein Sci. 4, 2411–2423.





**Protein Concentration Determination:** The concentration of RecA is between 1.9 and 2.1 mg/ml as determined by UV absorption at 280 nm by the Pace method using the extinction coefficient of 21,555 and molecular weight of 37,973 daltons (12).

**RecA Functional Assay (Triple Helix Formation):** The plasmid pUC19 contains 5 HpyCH4 IV sites. A 60 mer was designed with complementarity to the region centered around the HpyCH4 IV site at position 374. A reaction containing 1 µg pUC19, 0.18 µg 60 mer, 0.3 mM ATP γ-S, 4 µg RecA, in 40 µl 1X RecA Reaction Buffer was incubated at 37°C for 10 minutes to form a stable triple helix. The unprotected sites were methylated using 8 units of Sss I supplemented with 160 µM SAM for 10 minutes at 37°C. The reaction was stopped and the triple helix was disrupted by incubation at 65°C for 15 minutes. The reaction was cooled and 10 units of HpyCH4 IV were added followed by digestion at 37°C for 20 minutes. > 90% of the product is single cut pUC19.

**Heat Inactivation:** 65°C for 20 minutes.

Page 2 (M0249)

**References**:

1. West, S.C. (1992) *Ann. Rev. Biochem.* 61, 603–640.
2. Radding, C.M. (1991) *J. Biol. Chem.* 266, 5355–5358.
3. Wasserman, S.A. and Cozzarelli, N.R. (1985) *Proc. Natl. Acad. Sci. USA* 82, 1079–1083.
4. Shortle, D. et al. (1980) *Proc. Natl. Acad. Sci. USA* 77, 5375–5379.
5. Honigberg, S.M. et al. (1986) *Proc. Natl. Acad. Sci. USA* 83, 9586–9590.
6. Rigas, B. et al. (1986) *Proc. Natl. Acad. Sci. USA* 83, 9591–9595.
7. Ferrin, L.J. and Camerini-Otero, R.D. (1991) *Science* 254, 1494–1497.
8. Koob, M. et al. (1992) *Nucleic Acids Res.* 20, 5831–5836.
9. Koob, M. (1992). In R. Wu (Ed.), *Methods in Enzymology* Vol. 216, (pp. 321–329). San Diego: Academic Press.
10. Zhumabayeva, B. et al. (1990) *Biotechniques* 27, 834–845.
11. Zhumabayeva, B. et al. (2001) *Biotechniques* 30, 512–520.
12. Pace, C.N. et al. (1995) Protein Sci. 4, 2411–2423.

NEW ENGLAND BIOLABS® is a registered trademark of New England Biolabs, Inc.

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.