TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

Human DNA Polymerase β Kit

Catalog#: 4020-100-K

Contents:

Cat#	Description	Qty	Concentration
4020-100-01	Beta-polymerase	100 U	Lot specific
4020-050-02	Beta-pol Control DNA	10 µl	0.5mg/ml
3900-200-08	10X REC™ Buffer 8	1 ml	see below*
4020-050-04	Aphidicolin	10 µl	1 mM
4019-1	REC™ water	1 ml	N/A
4018-250	5X REC™ Loading buffer	250 µl	see below**

*10X REC™ Buffer 8 consists of 500 mM Tris-Cl (pH 8.8), 100 mM KCl, 100 mM MgCl , 10 mM DTT, 10% Glycerol

**20% Ficoll® 400; 20 mM EDTA, pH 8.0; 0.25% Bromophenol Blue.

Description: Human DNA Polymerase β is constitutively expressed in cells and functions by filling in gaps in DNA that are formed following base excision repair. The activity of DNA Polymerase β is not affected by aphidicolin, an inhibitor of DNA polymerases α, δ, and ε.

Source: Purified from *E. coli* containing a recombinant plasmid harboring the human DNA polymerase β gene.

Unit Definition: One Unit is the amount of enzyme required to catalyze the incorporation of 1 nmole of dNTP into an acid-insoluble form in 1 hour at 37°C.

Specificity: The enzyme can fill small gaps (up to 6 nucleotides) and nicks in DNA, catalyze DNA synthesis after nucleotide excision repair, and release 5'-terminal deoxyribose phosphate residues from incised AP sites.

Assay Conditions: 1X REC Buffer 8 (50 mM Tris-Cl (pH 8.8), 10 mM $MgCl_2$, 10 mM KCl, 1.0 mM DTT, 1% glycerol), 50 µM dCTP, 50 µM dGTP, 50 µM dATP, 50 µM α-^{32}P-dTTP, and 100 µg/ml of Activated DNA (Cat# 4667-50-06) in a reaction volume of 100 µl are incubated for 5 min at 37°C.

Storage Buffer: 20 mM Tris-Cl (pH 7.8), 1.0 mM DTT, 1 mM EDTA, 100 mM NaCl, and 50% (v/v) glycerol.

Storage Conditions: Store at -20°C in a manual defrost freezer.

TREVIGEN®
8405 Helgerman Court, Gaithersburg, MD 20877 USA Voice: 1-800-TREVIGEN (1-800-873-8443) • 301-216-2800
Fax: 301-560-4973 • e-mail: info@trevigen.com • www.trevigen.com

Application:

To use the β-polymerase enzyme, the following reaction can be set up with either radio-labeled α-^{32}P-dNTP or biotinylated nucleotides. The radiolabeled probe can be autoradiographed directly after electrophoresis, whereas the biotinylated nucleotide will need to be detected by Southern transfer of electrophoresed DNA, followed by Strep-HRP binding and colorimetric or chemiluminescent detection.

Reaction:

Sample or Control DNA		1 µl (0.5 µg)
10X REC™ Buffer 8		1 µl
β-polymerase enzyme		2-5 units (diluted in 1X Buffer)
α-^{32}P-dATP (3000 Ci/mMol)		10 pmol
10 mM each dCTP, dGTP, dTTP		1 µl
REC™ water		remainder
	Total	10 µl

Let the reaction proceed at 37°C for one hour, then add 2 µl of 5X REC™ Loading Buffer. Electrophorese the reaction products on a 0.8% TreviGel™ 5000/1X TAE gel until the blue dye has migrated 2/3 the distance of the gel, place onto a piece of 3MM paper, wrap in plastic wrap, (drying the gel down in a vacuum dryer is optional), then expose to auto-radiography.

The treated control DNA will generate a ladder with the following molecular weights: 23.13 Kbp, 9.4 Kbp, 6.6 Kbp, 4.4 Kbp, 2.3 Kbp, 2.0 Kbp, 0.5 Kbp.

Aphidicolin inhibits DNA polymerase α, but not β or γ. Therefore, it is useful to differentiate between different DNA polymerases.

References:

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