

Product:	Mega-Frag™ DNA Polymerase
Catalogue No:	CB-4010-5 250u CB-4010-7 500u
Description:	Mega-Frag is made up of a number of enzymes and additives, formulated for the purpose of research where high processivity and fidelity are needed. In the interest of obtaining the best possible results, included in the kit is a specially designed reaction buffer.
Storage buffer:	20mM Tris-HCl, pH 7.5, 100mM NaCl, 0.1mM EDTA, 2mM DTT, 50% Glycerol, and 0.1% Tween-20.
10x Reaction buffer:	Mega-Frag Buffer™
Mg⁺⁺ Stock solution:	50mM MgCl ₂
5x Specificity Enhancer	5x Additive. If necessary, 5x Additive should be re-dissolved by heating to 70°C and vortexing.
Unit definition:	One unit is defined as the amount that incorporates 10nmoles of dNTP's into acid-precipitable form in 30 minutes at 72°C under the standard assay conditions: 25mM TAPS (tris(hydroxymethyl)-methyl-amino-propanesulfonic acid, sodium salt) pH 9.3 (at 25°C), 50mM KCl, 2mM MgCl ₂ , 1mM β-mercapto-ethanol, 200μM each dATP, dGTP, dTTP, 100μM dCTP (a mix of unlabelled and α-[³² P] labelled); 12.5μg of activated salmon sperm DNA, in a final volume of 50μl.
Associated activities:	Endonuclease and exonuclease activities have not been detectable after 4 hours incubation of 1μg of pBR322 plasmid DNA and 0.5μg of HindIII-digested Lambda DNA at 72°C in the presence of 20 units of Mega-Frag.
Characteristics and applications:	<p>Long region: Mega-Frag is able to span the primer extension over long regions, thanks to the ideal composition of its different enzymatic activities. Furthermore, by reducing template degradation and premature termination of the strands, Mega-frag provides high processivity. It is possible to obtain >30kb products from λ templates, or 10kb products from genomic DNA, by using long primers at higher concentrations of Mg⁺⁺.</p> <p>High Performance: Impurities and other difficulties with templates do not impede Mega-Frag, which is designed to cope with problematic nucleotide compositions. Thus, Mega-Frag is able to operate with primers which are degenerate or do not match perfectly.</p> <p>Direct Cloning: Mega-Frag leaves an "A" overhang, which leads to the primer extension products being suitable for integrating effectively into TA cloning vectors, even in those instances where there are difficulties with templates. Mega-Frag is ideal for direct gene cloning, verification of the sequence prior to expression not being necessary.</p> <p>High Fidelity: Mega-Frag provides fidelity more than 7 times higher than Taq. This is achievable because it is a mix of polymerases with a 5' – 3' DNA polymerase activity and 3'-5' proof-reading activity, which during primer extension reactions, diminishes mis-incorporations. A further ingredient in Mega-Frag dispenses with the need for sequence-dependent removal of 3' terminal dNTPs in growing DNA chains (pyrophosphorolysis). Whereas most other proof-reading enzymes cause a degradation of primers, Mega-Frag does not do this.</p> <p>High Specificity: The Mega-Frag pack includes a "High Specification additive", which as the name implies, enhances specificity. This reagent, which is particularly valuable with difficult templates, helps to prevent false background bands from forming, and combats smearing. The concentration is decided on by the user on the basis of each experiment in question, but should be in the range 1-2 x final concentration. The ideal annealing temperature for primers may be affected when this additive is used, possibly necessitating some optimization.</p>
Suggestions and caution:	<ul style="list-style-type: none">Freezing, thawing and refreezing can cause the product to deteriorate.Elongation should be carried out at 68°CThe concentration of Mg should be optimized in steps of .2mM, starting from 2mM.Allow 0.06 sec./base if 4.0 units are used in a 50μl reaction volume.
Storage temperature:	The product should be stored in a constant-temperature freezer at -20°C. This will ensure its continued stability.
Batch details:	Batch No: See vial Units per vial: See vial Concentration: See vial

Denville Scientific Inc
PO Box 4588
Metuchen NJ 08840-4588
Tel: 908 757 7577
Fax: 908 757 7551
Email: info@denvillescientific.com

This certificate is a declaration of analysis at the time of manufacture