5581

Blend Taq

Code: TYB-BTQ-101 (250 units)

Storage: Store at -20℃

Concentration: 2.5 units/ul

Unit Definition: One unit of enzyme is defined as the amount of enzyme that will incorporate 10 nmoles of dNTPs into acid insoluble materials in 30 minutes at 75℃.

Assay Condition:

25	mM	TAPS (pH 9.3)
50	mM	KCl
10	mM	$MgCl_2$
200	uM	each of dATP, dGTP, dTTP
100	uM	[α-^{32}P]-dCTP
20	ug	activated salmon sperm DNA per 50 ul reaction

Storage Buffer:

20	mM	Tris-HCl (pH 8.0)
100	mM	KCl
0.1	mM	EDTA
1	mM	DTT
0.5	%	Tween-20
0.5	%	Nonidet P-40
50	%	Glycerol

Materials Provided: 10×Buffer for Blend Taq (Mg^{2+} concentration: 20mM)
dNTPs : 2mM dATP, dGTP, dCTP, dTTP each

Quality Control

1. PCR Assay: The 17.5kbp fragment of human β globin gene could be amplified using human genomic DNA.

PCR conditions

Reaction mixture for General PCR Amplification

10×Buffer for Blend Taq	5ul
Blend Taq (2.5 U/ul)	0.5ul (1.25U)
Template	1-50ng (plasmid)　10-1000ng (Genome)
Primer	10-50pmoles (final conc.: 0.2-1.0uM)
2mM dNTPs	5ul (final conc.: 0.2mM)
Total volume	50ul

Suggested Cycling parameters

<table>
<tr><th rowspan="2">Segment</th><th colspan="3">Target</th><th rowspan="2">Number of Cycles</th></tr>
<tr><th><1kb</th><th>1kb – 6kb</th><th>>6kb</th></tr>
<tr><td>1</td><td colspan="3">94℃
2min</td><td>1</td></tr>
<tr><td>2 Denaturation</td><td colspan="3">94℃
30sec (see notes #1)</td><td rowspan="3">30</td></tr>
<tr><td>3 Annealing</td><td colspan="2">Primer Tm-5℃ (55-60℃)
30sec</td><td rowspan="2">68℃
1min/kb PCR target
(see notes #2, #3)</td></tr>
<tr><td>4 Extension</td><td>72℃
1min</td><td>72℃
1min/kb PCR target</td></tr>
</table>

Notes

1. The denaturation time and temperature may require optimization. Typically denaturation time will range 25 - 30sec at 94℃ or 5 - 10sec at 98℃.
2. Toyobo suggests >70℃ melting temperature of primer (Tm) for long target (>6kb).
3. A dNTP concentration range of 0.3 - 0.4mM total is recommended for longer target (>10kb).
4. Blend Taq produces amplification products that are ready to clone directly into TA cloning vectors without any special optimization. To get sufficient numbers of transformants and white colony, longer ligation time will be efficient, recommended 2 hours – overnight for the amplification products of >1kb.
5. Blend Taq-Plus- is designed to be suitable for Hot Start PCR. Non-specific amplification due to mispriming and/or formation of primer dimer before thermal cycling can be prevented.

For research use only; not for use as a diagnostic.

Distributor
COSMO BIO CO., LTD.
Inspiration for Life Science
TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN
http://www.cosmobio.co.jp　e-mail : export@cosmobio.co.jp
Phone : +81-3-5632-9617　FAX : +81-3-5632-9618