
DIGEST LIKE A PRO: MANUAL

AKTIVITA ENZÝMU
DEFINÍCIA JEDNOTKY

One unit is defined as the amount of enzyme required to digest 1 µg of lambda DNA in 1 hour at 37°C in 50 µL of recommended reaction buffer

AKTIVITA ENZÝMU

1. závislá na čase
2. závislá na type substrátu
3. závislá na teplote
4. závislá na chem. faktoroch (pufri)
5. závislá na koncentrácii

ZÁVISLOSŤ NA ČASE
Classic vs. FastDigest

NdeI-Classic

- Incubate at 37°C for 1-16 hours.

NdeI-FD

Incubate at 37°C in a heat block or water thermostat for 5 min (plasmid DNA) or for 30 min (genomic DNA), or for ≥60 min (PCR product).

No detectable degradation of 1 µg of lambda DNA due to nuclease contamination or star activity occurred during incubation with 1 µL of FastDigest NdeI for 6 hours. Longer incubation may result in star activity.

ZÁVISLOSŤ NA ČASE/TYPE SUBSTRÁTU

BamHI-FD

- 1 µL of FastDigest BamHI is formulated to digest up to:
  - 1 µg of lambda DNA in 5 min.
  - 1 µg of plasmid DNA in 5 min.
  - 0.2 µg of PCR product in 5 min.
  - 1 µg of genomic DNA in 5 min, or 5 µg of genomic DNA in 30 min.

NdeI-FD

- 1 µL of FastDigest NdeI is formulated to digest up to:
  - 1 µg of lambda DNA in 5 min.
  - 1 µg of plasmid DNA in 5 min.
  - 0.2 µg of PCR product in ≥60 min.
  - 1 µg of genomic DNA in 30 min, or 5 µg of genomic DNA in 3 hours.

ZÁVISLOSŤ NA TYPE SUBSTRÁTU

Enzyme	Oligo Sequence	Chain Length	% Cleavage	
			2 hr	20 hr
NdeI	CCATATGG	8	0	0
	CCCATATGGG	10	0	0
	CGCCATATGGCG	12	0	0
	GGGTTTCATATGAAACCC	18	0	0
	GGAATTCCATATGGAATTCC	20	75	>90
	GGGAATTCCATATGGAATTCCC	22	75	>90

Enzyme	Base pairs from End	%Cleavage Efficiency	Vector	Initial Cut
HindIII	3	90	LITMUS 29	NcoI
	2	91	LITMUS 28	NcoI
	1	0	LITMUS 29	BamHI

ZÁVISLOSŤ NA TYPE SUBSTRÁTU

Prokaryotic Methylation

- Dam methylase–methylation at the N^6 position of the adenine in the sequence GATC (1,2).
- Dcm methyltransferases–methylation at the C5 position of the second cytosine in the sequences CCAGG and CCTGG (1,3).
- EcoKI methylase–methylation of adenine in the sequences AAC(N^6)GTGC and GCAC(N^6)GTT.

Some or all of the sites for a restriction endonuclease may be resistant to cleavage when isolated from strains expressing the Dam or Dcm methylases if the methylase recognition site overlaps the endonuclease recognition site. For example, plasmid DNA isolated from *dam+ E. coli* is completely resistant to cleavage by MboI, which cleaves at GATC sites.

Eukaryotic Methylation

CpG MTases, found in higher eukaryotes (e.g., Dnmt1), transfer a methyl group to the C^5 position of cytosine residues. Patterns of CpG methylation are heritable, tissue specific and correlate with gene expression. Consequently, CpG methylation has been postulated to play a role in differentiation and gene expression (4).

Enzyme	Cat #	Temp	% Activity in NEBuffer			
			1.1	2.1	3.1	CutSmart
BsaBI Yes dam	R0537	60°C	50	100	75	100

ZÁVISLOSŤ NA TEPLOTE/PUFRI

Enzyme	Cat #	Temp	% Activity in NEBuffer			
			1.1	2.1	3.1	CutSmart
BsaBI Yes dam	R0537	60°C	50	100	75	100
BamHI No	R0136	37°C	75*	100*	100	100*

Double Digest Recommendations for BsaBI + BamHI:

- Digest in NEBuffer 3.1 at 37°C with BamHI, then add BsaBI and raise temperature to 60°C.
- ** May exhibit star activity in this buffer.*

ZÁVISLOSŤ NA KONCENTRÁCII

Menej enzýmu za dlhší čas premení rovnaké množstvo substrátu.

Čiastočne degradovaný enzým po date of expiracy je nutné kompenzovať:

1. prídavkom počtu U enzýmu do reakcie
2. predĺženou dobou inkubácie

Štiepením over-night sa dá ušetriť vzácny enzým (pozor na star-activity)

ZDROJE

https://www.thermofisher.com

https://www.neb.com

https://www.neb.com/~/media/NebUs/Files/Chart%20image/cleavage_olignucleotides_old.pdf

https://www.neb.com/~/media/NebUs/Files/Chart%20image/cleavage_linearized_vector_old.pdf

https://www.neb.com/tools-and-resources/usage-guidelines/nebuffer-performance-chart-with-restriction-enzymes

https://www.neb.com/tools-and-resources/interactive-tools/double-digest-finder