2

Feb 10, 2022

Q5® Site-Directed Mutagenesis Kit Quick Protocol (E0554) V.2

New England Biolabs[1]

[1]New England Biolabs

1

dx.doi.org/10.17504/protocols.io.bddei23e

New England Biolabs (NEB)
Tech. support phone: **+1(800)632-7799** email: **info@neb.com**

New England Biolabs
New England Biolabs

This is the quick protocol for the Q5® Site-Directed Mutagenesis Kit (E0554).

DOI

dx.doi.org/10.17504/protocols.io.bddei23e

https://www.neb.com/protocols/2013/01/26/q5-site-directed-mutagenesis-kit-quick-protocol-e0554

New England Biolabs 2022. Q5® Site-Directed Mutagenesis Kit Quick Protocol (E0554). **protocols.io**
https://dx.doi.org/10.17504/protocols.io.bddei23e
New England Biolabs

site directed mutagenesis, exponential amplification for SDM, E0554, SDM

protocol ,

Mar 07, 2020

Feb 10, 2022

33926

MATERIALS

Q5 Site-Directed Mutagenesis Kit - 10 rxns **New England Biolabs Catalog #E0554S**

Please refer to Safety Data Sheets (SDS) for health and environmental hazards.

Primers should be designed with 5´ ends annealing back-to-back. We recommend using the NEB online design software, NEBaseChanger™.

Exponential Amplification (PCR)

1 

Assemble the following reagents in a thin-walled PCR tube.

A	B	C
	25 µl RXN	**FINAL CONC.**
Q5 Hot Start High-Fidelity 2X Master Mix	12.5 µl	1X
10 µM Forward Primer	1.25 µl	0.5 µM
10 µM Reverse Primer	1.25 µl	0.5 µM
Template DNA (1–25 ng/µl)	1 µl	1-25 ng
Nuclease-free water	9.0 µl	

2 

Mix reagents completely.

3 

Transfer to a thermalcycler and perform the following cycling conditions:

A	B	C
STEP	**TEMP**	**TIME**
Initial Denaturation	98°C	30 seconds
25 Cycles	98°C	10 seconds
	50–72°C*	10–30 seconds
	72°C	20–30 seconds/kb
Final Extension	72°C	2 minutes
Hold	4–10°C	

* For mutagenic primers, please use the Ta provided by the online NEB primer design software, NEBaseChanger™.

Kinase, Ligase & DpnI (KLD) Treatment

Citation: New England Biolabs Q5ÃÂ® Site-Directed Mutagenesis Kit Quick Protocol (E0554)
https://dx.doi.org/10.17504/protocols.io.bddei23e

4

Assemble the following reagents:

A	B	C
	VOLUME	**FINAL CONC.**
PCR Product	1 µl	
2X KLD Reaction Buffer	5 µl	1X
10X KLD Enzyme Mix	1 µl	1X
Nuclease-free Water	3 µl	

5

Mix well by pipetting up and down.

6

Incubate at **Room temperature** for **00:05:00** .

Transformation

7

Add **5 µL KLD mix** from previous step to **50 µL chemically-competent cells** .

8

Incubate **On ice** for **00:30:00** .

9 Heat shock at **42 °C** for **00:00:30** .

10

Incubate **On ice** for **00:05:00** .

11

Citation: New England Biolabs Q5ÃÂ® Site-Directed Mutagenesis Kit Quick Protocol (E0554)
https://dx.doi.org/10.17504/protocols.io.bddei23e

Add **950 µL SOC** .

12 Gently shake at **37 °C** for **01:00:00** .

13 

Spread **40 µL** – **100 µL** onto appropriate selection plate.

14 

Incubate **Overnight** at **37 °C** .

Citation: New England Biolabs Q5ÃÂ® Site-Directed Mutagenesis Kit Quick Protocol (E0554)
https://dx.doi.org/10.17504/protocols.io.bddei23e