QuikChange
Site-Directed Mutagenesis Kits

Find out how easy it is to upgrade your kit to QuikChange Lightning

- **Simple 3-Step Protocol**
- **>80% Mutation Efficiency**
- **Avoid Unintended Mutations**

The QuikChange Lightning Site-Directed mutagenesis and QuikChange Lightning Multi-Site Directed Mutagenesis Kits ramp up the QuikChange mutagenesis family with faster amplification and selection enzymes.

The accelerated protocols enable mutagenesis at a single site in less than half the time and at multiple sites (up to five) in a third of the time as our QuikChange and QuikChange II kits, while maintaining the same accuracy and mutation efficiency across an extensive range of plasmid types.

Additionally, the need to purchase separate kits for short and long templates for single site-directed mutagenesis has been eliminated: now, a single QuikChange Lightning kit can accommodate template sizes ranging from 4 to 14 Kb.

	SINGLE SITE-DIRECTED						MULTIPLE SITE-DIRECTED	
	QuikChange	**QuikChange XL**	**QuikChange II**	**QuikChange II-E**	**QuikChange II XL**	**QuikChange Lightning**	**QuikChange Multi**	**QuikChange Lightning Multi**
Catalog #	200518, 200519	200516, 200517	200523, 200524	200555	200521,200522	210518, 210519	200514, 200515, 200513, 200531	210513, 210515, 210514, 210516
# of Primers	2	2	2	2	2	2	1 Per Site; Up to 5	1 Per Site; Up to 5
Primer Type	Complementary Primer Pair	Complementary Primer Pair	Complementary Primer Pair	Complementary Primer Pair	Complementary Primer Pair	Complementary Primer Pair	1 Single-Stranded Primer Per Site	1 Single-Stranded Primer Per Site
Purified Primers	Recommended	Recommended	Recommended	Recommended	Recommended	Recommended	Recommended	Recommended
# Cycles	12-18	18	12-18	12-18	18	18	30	30
Polymerase	*PfuTurbo*	*PfuTurbo*	*PfuUltra*	*PfuUltra*	*PfuUltra*	QuikChange Lightning Enzyme	QuikChange Multi Enzyme Blend	QuikChange Lightning Multi Enzyme Blend
Type of Amplification	Linear Amplification						Linear Amplification	
Ability to Eliminate Unintended Mutations	Good	Good	Better	Better	Better	Best	Good	Best
DNA Template	Supercoiled, Dam-methylated						Supercoiled, Dam-methylated	
QuikSolution	No	Yes	No	No	Yes	Yes	Yes	Yes
Competent Cells	XL1-Blue Supercompetent	XL10-Gold Ultracompetent	XL1-Blue Supercompetent	XL1-Blue Supercompetent	XL10-Gold Ultracompetent	XL10-Gold Ultracompetent	XL10-Gold Ultracompetent	XL10-Gold Ultracompetent
Point Mutations	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Insertions/Deletions	Yes	Yes	Yes	Yes	Yes	Yes	No	No
Template Length (Kb)	4-8	8-14	4-8	4-8	8-14	4-14	4-8	4-8

QuikChange Lightning and QuikChange Kits Protocol Comparison – Single Site

Differences in protocols are highlighted in colors corresponding to each kit

Step 1 Prepare the control and sample reaction(s) as indicated below:

	QuikChange Lightning 10 to 100 ng dsDNA	QuikChange 5 to 50 ng dsDNA	QuikChangeXL 5 to 50 ng dsDNA
	Note: Set up a series of sample reactions using various amounts of dsDNA template ranging from 10 to 100 ng e.g.,10, 25, 50, and 100 ng.	**Note:** Set up a series of sample reactions using various amounts of dsDNA template ranging from 5 to 50 ng, e.g. 5, 10, 20 and 50 ng.	**Note:** Set up a series of sample reactions using various amounts of dsDNA template ranging from 5 to 50 ng, e.g. 5, 10, 20 and 50 ng.
Control Reaction	**5 µl of 10×** reaction buffer	**5 µl of 10×** reaction buffer	**5 µl of 10×** reaction buffer
	5 µl (25 ng) of pWhitescript 4.5 Kb control template (5 ng/µl)	**2 µl (10 ng)** of pWhitescript 4.5 Kb control template (5 ng/µl)	**2 µl (10 ng)** of pWhitescript 4.5 Kb control template (5 ng/µl)
	1.25 µl (125 ng) of control primer #1	**1.25 µl (125 ng)** of control primer #1	**1.25 µl (125 ng)** of control primer #1
	1.25 µl (125 ng) of control primer #2	**1.25 µl (125 ng)** of control primer #2	**1.25 µl (125 ng)** of control primer #2
	1 µl of dNTP mix	**1 µl** of dNTP mix	**1 µl** of dNTP mix
	34 µl ddH_2O to a final volume of 50 µl	**38.5 µl** ddH_2O to a final volume of 50 µl	**36.5 µl** ddH_2O to a final volume of 50 µl
	1.5 µl of QuikSolution reagent	——	**3 µl** of QuikSolution reagent
Sample Reaction	**5 µl of 10×** reaction buffer	**5 µl of 10×** reaction buffer	**5 µl of 10×** reaction buffer
	X µl (10-100 ng) of dsDNA template	**X µl (5-50 ng)** of dsDNA template	**X µl (10 ng)** of dsDNA template
	X µl (125 ng) of oligonucleotide primer #1	**X µl (125 ng)** of oligonucleotide primer #1	**X µl (125 ng)** of oligonucleotide primer #1
	X µl (125 ng) of oligonucleotide primer #2	**X µl (125 ng)** of oligonucleotide primer #2	**X µl (125 ng)** of oligonucleotide primer #2
	1 µl of dNTP mix	**1 µl** of dNTP mix	**1 µl** of dNTP mix
	ddH_2O to a final volume of 50 µl	ddH_2O to a final volume of 50 µl	ddH_2O to a final volume of 50 µl
	1.5 µl of QuikSolution reagent	——	**3 µl** of QuikSolution reagent

Step 2 Add **1 µl** QuikChange Lightning Enzyme / *PfuTurbo* DNA Polymerase (2.5 U/µl) / *PfuTurbo* DNA Polymerase (2.5 U/µl) to each control and sample reaction

Step 3 Cycle each reaction using the cycling parameters outlined in the following table:

Segment	Cycles	Temperature	Time
1	1 / 1 / 1	95 °C / 95 °C / 95 °C	2 minutes / 30 seconds / 1 minute
2	18	95 °C / 60 °C / 68 °C	20 seconds / 10 seconds / 30 seconds*
	12–18	95 °C / 55 °C / 68 °C	30 seconds / 1 minute / 1 minute*
	18	95 °C / 60 °C / 68 °C	50 seconds / 50 seconds / 1 minute*
3	1 / no cycle needed / 1	68 °C / no cycle needed / 68 °C	5 minutes / no cycle needed / 7 minutes

*/Kb of plasmid length

QC only: Adjust segment 2 of the cycling parameters in accordance with the type of mutation desired. (See details in manual.)

Step 4 Add 2 µl / 1 µl / 1 µl of the *Dpn* I restriction enzyme

Step 5 Gently and thoroughly mix each reaction, microcentrifuge briefly, then immediately incubate at **37°C** for 5 minutes / 1 hour / 1 hour to digest the parental dsDNA

Step 6 Transform 2 µl / 1 µl / 2 µl of the *Dpn* I-treated DNA from each reaction into separate 45 µl / 50 µl / 45 µl aliquots of XL10-Gold Ultracompetent / XL1-Blue Supercompetent / XL10-Gold Ultracompetent cells

(see Transformation of Competent Cells in the instruction manual)

QuikChange Lightning and QuikChange II Kits Protocol Comparison – Single Site

Differences in protocols are highlighted in colors orresponding to each kit.

Step 1 Prepare the control and sample reaction(s) as indicated below:

	QuikChange Lightning 10 to 100 ng dsDNA	QuikChange II 5 to 50 ng dsDNA	QuikChange II XL 5 to 50 ng dsDNA
	Note: Set up a series of sample reactions using various amounts of dsDNA template ranging from 10 to 100 ng, e.g., 10, 25, 50, and 100 ng.	**Note:** Set up a series of sample reactions using various amounts of dsDNA template ranging from 5 to 50 ng, e.g., 5, 10, 20, and 50 ng.	**Note:** Set up a series of sample reactions using various amounts of dsDNA template ranging from 5 to 50 ng, e.g., 5, 10, 20, and 50 ng.
Control Reaction	**5 µl of 10×** reaction buffer	**5 µl of 10×** reaction buffer	**5 µl of 10×** reaction buffer
	5 µl (25 ng) of pWhitescript 4.5 Kb control template (5 ng/µl)	**2 µl (10 ng)** of pWhitescript 4.5 Kb control template (5 ng/µl)	**2 µl (10 ng)** of pWhitescript 4.5 Kb control template (5 ng/µl)
	1.25 µl (125 ng) of control primer #1	**1.25 µl (125 ng)** of control primer #1	**1.25 µl (125 ng)** of control primer #1
	1.25 µl (125 ng) of control primer #2	**1.25 µl (125 ng)** of control primer #2	**1.25 µl (125 ng)** of control primer #2
	1 µl of dNTP mix	**1 µl** of dNTP mix	**1 µl** of dNTP mix
	34 µl ddH_2O to a final volume of 50 µl	**38.5 µl** ddH_2O to a final volume of 50 µl	**35.5 µl** ddH_2O to a final volume of 50 µl
	1.5 µl of QuikSolution reagent	——	**3 µl** of QuikSolution reagent
Sample Reaction	**5 µl of 10×** reaction buffer	**5 µl of 10×** reaction buffer	**5 µl of 10×** reaction buffer
	X µl (10–100 ng) of dsDNA template	**X µl (5-50 ng)** of dsDNA template	**X µl (10 ng)** of dsDNA template
	X µl (125 ng) of oligonucleotide primer #1	**X µl (125 ng)** of oligonucleotide primer #1	**X µl (125 ng)** of oligonucleotide primer #1
	X µl (125 ng) of oligonucleotide primer #2	**X µl (125 ng)** of oligonucleotide primer #2	**X µl (125 ng)** of oligonucleotide primer #2
	1 µl of dNTP mix	**1 µl** of dNTP mix	**1 µl** of dNTP mix
	ddH_2O to a final volume of 50 µl	ddH_2O to a final volume of 50 µl	ddH_2O to a final volume of 50 µl
	1.5 µl of QuikSolution reagent	——	**3 µl** of QuikSolution reagent

Step 2 Add **1 µl** QuikChange Lightning Enzyme / *PfuUltra* HF DNA Polymerase (2.5 U/µl) / *PfuUltra* HF DNA Polymerase (2.5 U/µl) to each control and sample reaction

Step 3 Cycle each reaction using the cycling parameters outlined in the following table:

Segment	Cycles	Temperature	Time
1	1 / 1 / 1	95 °C / 95 °C / 95 °C	2 minutes / 30 seconds / 1 minute
2	18	95 °C / 60 °C / 68 °C	20 seconds / 10 seconds / 30 seconds*
	12–18	95 °C / 55 °C / 68 °C	30 seconds / 1 minute / 1 minute*
	18	95 °C / 60 °C / 68 °C	50 seconds / 50 seconds / 1 minute*
3	1 / no cycle needed / 1	68 °C / no cycle needed / 68 °C	5 minutes / no cycle needed / 7 minutes

*/Kb of plasmid length

QC II only: Adjust segment 2 of the cycling parameters in accordance with the type of mutation desired. (See details in manual.)

Step 4 Add 2 µl / 1 µl / 1 µl of the *Dpn* I restriction enzyme

Step 5 Gently and thoroughly mix each reaction, microcentrifuge briefly, then immediately incubate at **37°C** for 5 minutes / 1 hour / 1 hour to digest the parental supercoiled dsDNA

Step 6 Transform 2 µl / 1 µl / 2 µl of the *Dpn* I-treated DNA from each reaction into separate 45 µl / 50 µl / 45 µl aliquots of XL10-Gold Ultracompetent / XL1-Blue Supercompetent / XL10-Gold Ultracompetent cells
(see Transformation of Competent Cells in the instruction manual)

QuikChange Lighting Multi and QuikChange Multi Kits Protocol Comparison – Multiple Sites

Differences in protocols are highlighted in colors corresponding to each kit.

Step 1 Prepare mutant strand synthesis reaction(s) in thin-walled tubes as indicated below:

	QuikChange Lightning Multi	QuikChange Multi
Control Reaction	**2.5 µl 10×** reaction buffer	**2.5 µl 10×** reaction buffer
	1 µl control template	**1 µl** control template
	1 µl control primer mix	**1 µl** control primer mix
	1 µl dNTP mix	**1 µl** dNTP mix
	1 µl QuikChange Lightning Multi enzyme	**1 µl** QuikChange Multi enzyme
	18.5 µl ddH2O	**18.5** µl ddH2O
Mutagenesis Reaction	**2.5 µl 10×** Reaction Buffer	**2.5 µl 10×** Reaction Buffer
	X µl ds-DNA template (50ng for ≤5 Kb, or 100 ng for >5 Kb*)	**X µl** ds-DNA template (50ng for ≤5 Kb, or 100 ng for >5 Kb*)
	X µl each primer (100 ng each for 1-3 primers or 50 ng each for 4-5 primers)	**X µl** each primer (100 ng each for 1-3 primers or 50 ng each for 4-5 primers)
	1 µl dNTP mix	**1 µl** dNTP mix
	1 µl QuikChange Lightning Multi enzyme	**1 µl** QuikChange Multi enzyme
	ddH_2O to a final volume of 25 µl	ddH_2O to a final volume of 25 µl
	* For templates >5 Kb, also add 0–0.75 µl QuikSolution to the reaction (titrate the QuikSolution to determine optimal amount).	* For templates >5 Kb, also add 0–0.75 µl QuikSolution to the reaction (titrate the QuikSolution to determine optimal amount).

Step 2 Add **1 µl** of QuikChange Lightning Enzyme / QuikChange Multi Enzyme to each control and sample reaction

Step 3 Cycle each reaction using the cycling parameters outlined in the following table:

Segment	Cycles	Temperature	Time
1	1 / 1	95 °C / 95 °C	2 minutes / 1 minute
2	30	95 °C / 55 °C / 65 °C	20 seconds / 30 seconds / 30 seconds*
	30	95 °C / 55 °C / 65 °C	1 minute / 1 minute / 2 minutes*
3	1 / no cycle needed	65 °C / no cycle needed	5 minutes / no cycle needed

*/Kb of plasmid length

Step 4 Add 1 µl / 1 µl of the *Dpn* I restriction enzyme

Step 5 Gently and thoroughly mix each reaction, spin down in a microcentrifuge for 1 minute, and immediately incubate at **37°C** for 5 minutes/ 1 hour to digest parental DNA

Step 6 Transform **1.5 µl** of the *Dpn* I-treated DNA into a 45-µl aliquot of XL10-Gold Ultracompetent Cells

(see Transformation of XL10-Gold Ultracompetent Cells in the instruction manual)

For more information

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