MFC Mutagenesis Kit

(Multi-Fast-Change Mutagenesis Kit)

INSTRUCTION MANUAL

(for CAT#: MFC-20-A, MFC-20-B, MFC-100-A, MFC-100-B)

MFC Mutagenesis Kit (Multi-Fast-Change Mutagenesis Kit) provides a fast, highly efficient, and cost-effective site-directed mutagenesis method with high fidelity master mix to introduce single or multiple mutations (such as base pair changes, insertions, and deletions). Specially, only 1 primer per mutation site is needed. Up to 5 sites can be mutagenized in a single reaction.

Kit components

("A" includes *Dpn*I and competent cells, "B" has the basic master mix only**)**

Materials provided	MFC-20-A	MFC-20-B	MFC-100-A	MFC-100B
2x MFC master mix*	250 µL	250 µL	250 µL X 5	250 µL X 5
*Dpn*I restriction enzyme**	20 µL	-	100 µL	-
DH5alpha Competent cells ***	20 x 50 µL		100 x 50 µL	-

* Each 25 µL reaction uses 12.5 µL 2x MFC high-fidelity master mix.

** Each 25 µL reaction uses 1 µL *Dpn*I restriction enzyme.

*** Could also use customer's own competent cells for MFC-20-A and MFC-100-A.

Primer Design

- **Only a single primer is required** for each mutation site. Either sense or anti-sense strand can be used for primer design.
- For multiple mutation sites, make sure all primers are in the same orientation, and they are also not overlapping with each other.
- Please follow the general guidelines for design of mutagenesis primers. It is recommended to have at least 15 nt on each side of the point mutation, and at least 18-21 nt on each side for multiple base pair changes, insertion, and deletion mutagenesis.

Protocols

1. Prepare the MFC mutagenesis reactions following the table below:

	Mutagenesis Reaction
2x MFC master mix	12.5 µL
Plasmid DNA (~100-200 ng/µL)	1.0 µL
Mutagenesis Primer (10 µM) *	1.0 µL
Add Nuclease free H2O to	**25 µL**

* Up to 5 different primers can be mixed in the same reaction for combining mutations at multiple sites onto the same DNA strand.

2. Set up the reactions on a thermal cycler using the following programs:

	Temperature	**Time**
Initial denaturation	98°C	2 min
Cycling (x30)	98°C	15 sec
	55-60°C*	30 sec
	72°C	30 sec per kb
Final extension	72°C	7 min
Holding	14°C	∞

* The annealing temperature depends on the specific sequence of the customer primer, which can be calculated following the general rules of site-directed mutagenesis primers.

3. Treat the mutagenesis reaction with *Dpn*I :
    a. For cat# **MFC-20-B and MFC-100-B**, please follow supplier's protocol
    b. For cat# **MFC-20-A and MFC-100-A**:
        i. Add 1 µL ***Dpn*I restriction enzyme** per reaction
        ii. Mix well via pipetting up and down
        iii. Incubate at 37°C for 30 min, then put the reaction on ice

4. Transform *Dpn*I-digested mutagenesis reaction into *E. coli* competent cells:
    a. For cat# **MFC-20-B and MFC-100-B**, please follow supplier's protocol
    b. For cat# **MFC-20-A and MFC-100-A**:
        i. Add 2-5 µL reaction into a vial of 50 µL **DH5alpha Competent cells**, mixing with gentle tapping
        ii. Incubate on ice for 30 min
        iii. Heat-shock the cells at 42°C for 30 sec
        iv. Incubate on ice for 2 min
        v. Add pre-warmed 450 µL SOC to each vial
        vi. Incubate the vials at 37°C for 1 hour with shaking at 225–250 rpm
        vii. Plate 50-100 µL of each transformation reaction on agar selection plates with the appropriate antibiotic
        viii. Incubate the transformation plates at 37°C overnight

5. Pick colonies and culture, then miniprep and sequence to identify positive clones.