SGIDNA

Gibson Assembly® Site-Directed Mutagenesis Kit

Quick Reference Manual for Experienced Users

For additional product information, an online SDM primer design tool, and detailed protocol visit **sgidna.com/sdm-kit**

Catalog Numbers GA2100-S and GA2100-10

Products and Storage Conditions

Gibson Assembly® Site-Directed Mutagenesis Kit Contents

Quantity	Component	Cat. GA2100-S (5 Reactions)	Cat. GA2100-10 (10 Reactions)	Storage Temperature
		Volume		
1 tube (each)	GA SDM PCR Mix (2X)	315 µL	625 µL	–20°C
	GA SDM Assembly Mix A (2X)*	25 µL	50 µL	
	GA SDM Assembly Mix B (2X)*	50 µL	100 µL	
	Control Plasmid (5 ng/µL)	5 µL (2 Control Rxns)	5 µL (2 Control Rxns)	
	Control Primer Mix A (25 µM)	5 µL	5 µL	
	Control Primer Mix B (25 µM)	5 µL	5 µL	

*Aliquot the Assembly Mixes to reduce the number of freeze-thaw cycles.

Mutagenesis Amplification Reaction Setup

1. Thaw GA SDM PCR Mix (2X) on ice.
2. For each fragment, prepare the following reaction mixture in a thin-walled PCR tube on ice:

Component	Volume
GA SDM PCR Mix (2X)	12.5 µL
dsDNA template (100 pg – 30 ng)	χ µL
SDM Forward Primer (5 µM)	2.5 µL
SDM Reverse Primer (5 µM)	2.5 µL
PCR water	7.5–χ µL
Total	to 25 µL

3. *(Optional)* For the positive control, prepare the following reaction mixtures in thin-walled PCR tubes. Set up one reaction for Control Amplicon A and set up a separate reaction for Control Amplicon B (i.e. use Control Primer Mix A in one reaction and Control Primer Mix B in a separate reaction.)

Component	Volume
GA SDM PCR Mix (2X)	12.5 µL
Control Plasmid (5 ng/ µL)	1.0 µL
Control Primer Mix A or B (25 µM)	1.0 µL
PCR water	10.5 µL
Total	25 µL

4. Mix by pipetting up and down.

Mutagenesis Amplification Reaction

1. Transfer tubes to a thermocycler. Program and start the thermocycler using the following conditions:

Denature	98°C	1 min	1 Cycle
Amplify	98°C	10–30 sec	30 Cycles
	Primer T_m*	30 sec	
	72°C	30 sec per kb	
Extend	72°C	5 min	1 Cycle
Hold	4°C	as necessary	1 Cycle

*Control Primers T_m is 60°C.

2. After the reaction is complete, analyze 5 µL of the product on a 0.8% agarose gel.
3. Minimize original template carryover by:
   - Gel-purifying the PCR product

     or
   - Treating the PCR product with DpnI and column-purifying the DpnI-treated reaction.

Gibson Assembly® Reaction Setup

1. Thaw GA SDM Assembly Mix A (2X) on ice.
2. Adjust the concentration of the purified, mutagenized fragments according to the following table. In a PCR tube, combine the insert(s) and vector, bringing the total volume to 5 µL with nuclease-free water.

Fragment	Size	Amount	Insert:Vector Molar Ratio
Insert	≤ 1 kb	10–40 ng	1:1 to 5:1
	1–5.5 kb	10–25 ng	1:1 to 2:1
Vector	2–10 kb	25 ng	—

Note: For dilute fragments, you may increase the total combined sample volume to 10 µL. For 10 µL samples, increase subsequent assembly volumes 2-fold.

3. *(Optional)* For the positive control, combine 25 ng of gel- or column-purified Positive Control Amplicon A with 25 ng of gel- or column-purified Positive Control Amplicon B. Bring the total volume to 5 µL with nuclease-free water.
4. Vortex GA SDM Assembly Mix A (2X) immediately before use (after it is thawed).
5. In a tube on ice, combine 5 µL of volume-adjusted, purified mutagenized fragments and 5 µL of thawed GA SDM Assembly Mix A (2X).
6. Mix the reactions by pipetting up and down.
7. Vortex and spin down all reactions. Proceed to the Gibson Assembly® Reaction.

The Gibson Assembly® Reaction

1. Transfer tubes to a thermocycler. Program and start the thermocycler using the following conditions (1 cycle):

3′ end chew back	37°C for 5 minutes
Inactivation	75°C for 20 minutes
Slow cooling	0.1°C/second to 60°C
Annealing	60°C for 30 minutes
Slow cooling	0.1°C/second to 4°C

2. Thaw GA SDM Assembly Mix B (2X) on ice. **Vortex and briefly centrifuge the thawed master mix immediately before use.**
3. While keeping the tubes on ice, add 10 µL of GA SDM Assembly Mix B (2X) to the completed reaction from step 1.
4. Mix the reaction by pipetting.
5. Incubate samples using the following conditions:

Repair	1 cycle	45°C for 15 minutes

6. Store completed reactions at –20°C or proceed to downstream applications (e.g. transformation).
7. *(Optional)* Electrophorese 50% of the assembly reaction (e.g. 10 µL of a 20 µL reaction) on a 0.8–2% agarose gel. A high molecular weight smear is indicative of a successful assembly reaction.

Transformation Guidelines

For electrocompetent cells

- Use 1–2.5 uL of Gibson Assembly® Reaction per 30–50 uL of competent cells.
- Use high efficiency competent cells (transformation efficiency ≥1 × 10^9 CFU/µg pUC19). We recommend TransforMax™ EPI300™ Electrocompetent *E. coli* (Epicentre® Cat. No. EC300110)

For chemically competent cells

- Use 1 uL of Gibson Assembly® Reaction per 50 uL of competent cells.
- Use high efficiency competent cells (transformation efficiency ≥1 × 10^9 CFU/µg pUC19). We recommend *E. cloni* 10G Chemically Competent Cells (Lucigen Cat. No. 60107)

Plating Guidelines

Following transformation, plate 1/2–1/50 of the transformation reaction onto an LB plate containing appropriate antibiotics. We recommend plating 2 volumes. For the positive control, plate 1/100 volume of the transformed reaction onto an LB plate with 100 µg/mL ampicillin or carbenicillin and 40 µg/mL X-Gal and 0.1 mM IPTG.

Additional product information and resources are available at sgidna.com/sdm-kit

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