Gibson Assembly® RapidAMP™ Ultra Kit

Quick Reference Manual

Catalog Numbers GA1220-S, GA1220-10, GA1220-50

Products and Storage Conditions

Gibson Assembly® RapidAMP™ Ultra Kit

Quantity	Component	Cat. GA1220-S (5 Reactions)	Cat. GA1220-10 (10 Reactions)	Cat. GA1220-50 (50 Reactions)	Storage Temperature
		Volume			
1 tube each (except RapidAMP 50rxns)*	GA Ultra Master Mix A (2X)	25 µL	50 µL	250 µL	−20°C
	GA Ultra Master Mix B (2X)	50 µL	100 µL	500 µL	
	RapidAMP Master Mix (3X)	50 µL	100 µL	5 X 100 µL	
	GA Positive Control (2X)	10 µL (2 Control Rxns)	10 µL (2 Control Rxns)	25 µL (5 Control Rxns)	

*50 reaction kit has pre-aliquoted tubes for additional stability

Important Design Note Before Starting

Most customers will want to linearize with a single restriction enzyme cut in a non-essential portion of their backbone. **We recommend selecting a suitable cut-site for restriction digestion prior to gene synthesis** and ensure there are no other recognition sequences in the gene to be synthesized. It is necessary to include this restriction site in the initial construct design.

Guidelines

- For a typical Gibson Assembly® Ultra reaction, combine 25–50 ng of vector with 25–300 ng of insert. For best results, we recommend balancing the molar ratio of the DNA fragments. For fragments >1 kb, use an equimolar ratio. For DNA fragments ≤1 kb, we recommend using a 5-fold molar excess of insert. Refer to the table on page 2 for approximate pmol of DNA based on the size and amount of a given fragment. To precisely determine the pmol or ng of DNA based on fragment size, use the following formulas:

  pmol DNA = [ng DNA/(660 x # of bases)] x 1000
  ng of DNA = [pmol DNA x (660 x # of bases)]/1000

- The total volume for the combined DNA fragments in the assembly reaction is ≤ 5 µL.
- To assemble multiple fragments and minimize pipetting error, create a master mix of fragments in the proper ratios.

Gibson Assembly Ultra Procedure

1. Thaw GA Ultra Master Mix A (2X) on ice.
2. Dilute DNA fragments with nuclease-free water in PCR tubes to a total volume of 5 µL.
3. **Vortex the thawed master mix immediately before use.**
4. In a 0.2 mL PCR tube on ice, combine 5 µL of DNA fragments and 5 µL of GA Ultra Master Mix A (2X). Mix the reaction by pipetting.
5. (Optional) Set up a positive control reaction by aliquoting 5 µL of GA Positive Control (2X) into a 0.2 mL PCR tube on ice. Add 5 µL of GA Ultra Master Mix A (2X) and mix the reaction by pipetting. Spin down all reactions.

Gibson Assembly Ultra Procedure Continued

6. Program the following conditions into a thermocycler.

3' end Chew back	37°C for 5 min
Inactivation	75°C for 20 min (for all overlap sizes)
Slowly Cool	0.1°C/sec to 60°C
Anneal	60°C for 30 min
Slowly Cool	0.1°C/sec to 4°C

7. Transfer assembly tubes to the thermocycler and start.
8. Thaw GA Ultra Master Mix B (2X) on ice and **vortex the thawed master mix immediately before use.**
9. While keeping tubes on ice, add 10 µL of GA Ultra Master Mix B (2X) to the reactions from step 7. Mix the reaction by pipetting.
10. Incubate the reactions using the following conditions:

Repair	45°C for 15 min

11. After the incubation is complete, store reactions at −20°C or proceed to RapidAMP Amplification Step.

RapidAMP Amplification Procedure

1. Thaw RapidAMP Master Mix (3X) on ice.
2. **Vortex the thawed master mix immediately before use.**

   Note: For best results, do not freeze thaw tubes more than 5 times.
3. In the same 0.2 mL PCR tube from the Gibson Assembly reactions, on ice, add 10 µL of RapidAMP Master Mix (3X) to the 20 µL of Gibson Assembly Ultra reaction product. Mix the reaction by pipetting. Spin down all reactions.
4. Incubate the reactions using the following conditions:

Amplification	30°C for 4 hours
Heat Inactivate	65°C for 20 min

5. After the incubation is complete, store reactions at −20°C or proceed to **linearization via restriction digestion**.

Guidelines for Linearization

We recommend performing a Restriction Digestion protocol after completion of the RapidAMP procedure. The final amplified product is concatenated and highly branched. **For best results, reduce to linear monomers by restriction digestion.**

For optimal digestion, dilute the product 1:1 with the restriction enzyme mix (see below). However, the product can be kept at a higher concentration by adding less volume, increasing the digestion incubation time, and vortexing occasionally during the incubation to ensure efficient digestion. The amplified solution may be viscous. You may use your preferred restriction digestion protocol* or follow the generalized directions below.

***Note:** For complete DNA digestion, incubation times may need to increase due to the high yield of amplified DNA.

Linearization via Restriction Digest Procedure

1. Dilute 30 µl of RapidAMP final product 1:1 with 30 µl water (total 60 µl).

   Note: Optional quantification step here with Qubit for dsDNA.
2. Optional: If you need to keep a portion of RapidAMP as undigested, such as 10 µl, then transfer 50 µl 2X diluted amplified product to a new tube for the digestion reaction.
3. Add equal volume (50 µl or 60 µl) of 1X restriction digest buffer (final buffer concentration will be 0.5X, accounting for presence of buffers in previous steps) and 2 µl restriction enzyme to the tube with 2X diluted RapidAMP product.
4. Digest at 37°C for at least 2 hours. The digestion reaction can be scaled down or up with the same ratio as needed.

   Note: Typical restriction enzyme from NEB is 20,000 units/ml. If using restriction enzyme with less units/ml, adjust the enzyme volume according to the enzyme units.

   More restriction enzyme can also be added if shorter digestion time is preferred. However, don't use more than 5µl enzyme (no more than 1 µl enzyme per 20 µl digestion). Digestion reactions can also go up to overnight, depending on the DNA input and amount of enzyme in the reaction.
5. Heat inactivate the enzyme before going directly into transfection or some other cell-based assay.

Quantify Linearized DNA Yield (3 options)

- **Qubit**: Make a 2X dilution: Mix 5 µl of digestion reaction with 5 µl water, and then use 2 µl for BR dsDNA Qubit. The final concentration equals qubit concentration * dilution factor (1:8).
- **Densitometry:** Run gel with 2 µl diluted digestion and use densitometry.
- **Nanodrop:** Clean up via bead-based or column purification and nanodrop.

Optional Further DNA Processing

Purify digested reaction: For transient transfection, either digested and heat-inactivated DNA (straight off step #5 in linearization procedure) or DNA that has been further purified to remove previous assay components will be effective. However, for sensitive cell-based applications as well as *in vitro* transcription, DNA clean-up has been shown to increased performance. We recommend bead-based purification, such as AMPure, to minimize loss of DNA during purification. Column purification is also acceptable although more DNA may be lost.

Circularize monomers: In addition, many customers would prefer to work with circularized final constructs. To do this, after restriction digestion, the linear product must be phosphorylated with a kinase and then ligated. Optimization maybe be required.

Optional Transformation and Sequencing

RapidAMP amplification utilizes the high-fidelity Phi29 DNA Polymerase and should not introduce new errors when starting from an error-free circular template. However, for those who want to perform RapidAMP amplification, but require a homogenous DNA mix for later applications like stable transfection, an intermediate transformation and sequencing step would be required.

For these customers, the Gibson Assembly RapidAMP Ultra kit is still useful, but transformation and sequencing will be necessary for the Gibson Assembly product. We recommend diluting the assembly reaction 1:2 with water and transforming into DH5α *E. coli* cells for high transformation efficiency. Depending on the number and size of fragments, picking 2-8 colonies for sequencing should be sufficient. Once an error-free clone has been identified, it can be used as a template for RapidAMP amplification. Despite the addition of these intermediate steps, RapidAMP will still negate the need for multiple bacterial minipreps or maxipreps to acquire sufficient DNA for downstream applications.

Reference Material

Amount of DNA to use in Gibson Assembly® reaction

Refer to the following table for approximate pmol of DNA for a given fragment size and amount.

Fragment size	ng of DNA	pmol of DNA
0.4 - 1 kb	20 ng	0.061
	40 ng	0.121
1 - 5 kb	10 ng	0.015
	25 ng	0.038
5 - 8 kb	10 ng	0.003
	25 ng	0.008
8 - 10 kb	25 ng	0.005
	50 ng	0.009

Technical Services	For technical assistance, please contact technical services at techservices@sgidna.com.
Trademark Information	Gibson Assembly® is a registered trademark of SGI-DNA, Inc.
Label License Research Use Only	Except as otherwise agreed in writing by our authorized representative, this product is for INTERNAL RESEARCH USE ONLY AND NOT FOR HUMAN, ANIMAL, THERAPEUTIC OR DIAGNOSTIC USE. For additional information about your rights under this research license, please see our website at sgidna.com.
Disclaimer	The material in this manual is for informational purposes only and is subject to change without prior notice at any time. SGI-DNA and/or its affiliates assume no responsibility for any errors that may appear in this document.

REV.1.0 11.18.19

PN 40058

SGI-DNA, Inc.