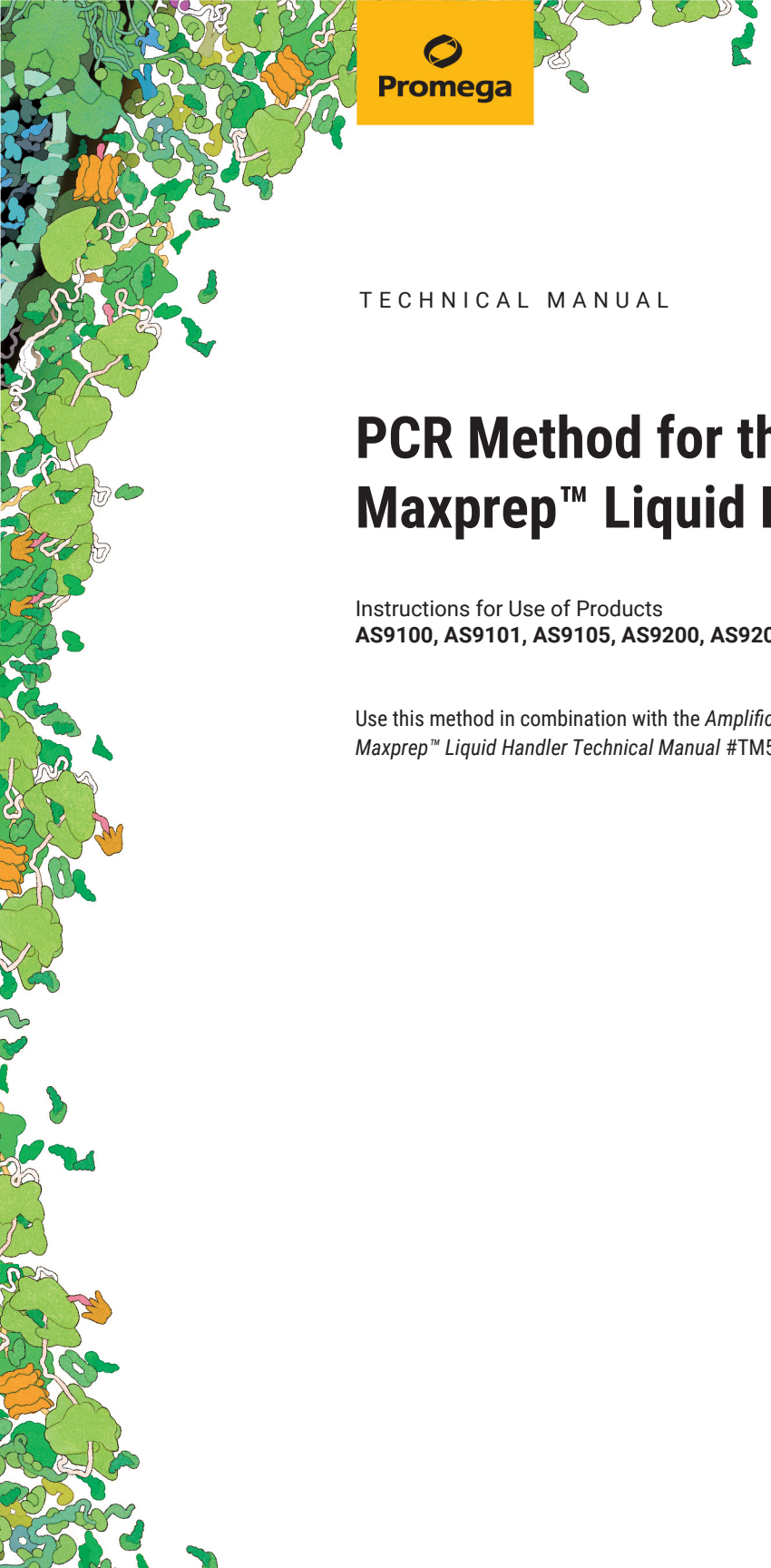

TECHNICAL MANUAL

PCR Method for the Maxprep™ Liquid Handler

Instructions for Use of Products
AS9100, AS9101, AS9105, AS9200, AS9201 and AS9205

Use this method in combination with the *Amplification Setup Methods for the Maxprep™ Liquid Handler Technical Manual* #TM526

Revised 1/24
TM635

PCR Method for the Maxprep™ Liquid Handler

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The PCR Method for the Maxprep™ Liquid Handler is designed to automate preparation of endpoint and quantitative PCR and RT-PCR amplifications, and includes master mix preparation, serial dilution of standard curves, sample dilution and control placement. Administrators can create variant methods in the Maxprep™ software that specify reaction setup options to meet the needs of the laboratory.

2. Materials to Be Supplied by the User

- amplification plate(s), user-specified
- 1.5ml tubes (e.g., ClickFit Microtube, 1.5ml, Cat.# V4741)
- Maxprep™ 3-Position Reagent Tube Holders (Cat.# AS9409) [up to 3 needed, depending on variant settings]
- Maxprep™ 50µl Conductive Disposable Tips, Filtered (Cat.# AS9301)
- Maxprep™ 300µl Conductive Disposable Tips, Filtered (Cat.# AS9302)
- **optional:** strip tubes, user-specified
- **optional:** 1.1ml, Square-Well V-Bottom Deep Well Plate (Cat.# V6821)
- **optional:** amplification plate base (e.g., Thermo Fisher Scientific MicroAmp® 96-well base, Cat.# N8010531)
- **optional:** Maxprep™ Reagent Reservoir, 50ml (Cat.# AS9304)

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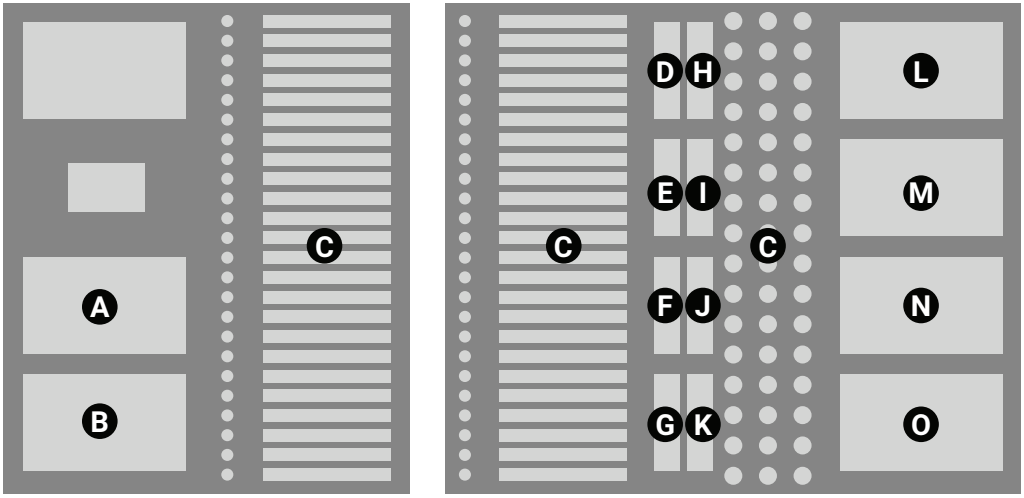
3. Run-Specific Information

The first screen of the method requests information regarding general method run parameters such as input labware, destination labware and whether to perform a 100-fold dilution of samples prior to amplification setup.

1. Use the drop-down menus to specify the Input Labware and Destination Labware; samples are placed on the system in the Input Labware, and the amplification reaction is prepared in the Destination Labware. Maxwell® deck trays, plates and tubes can all be used as input labware options for amplification setup.
2. Check 'Dilute Samples 100-fold' to dilute both samples and controls 100-fold prior to amplification setup.
3. The top right corner of the screen shows the Maximum Number of Samples allowed within this amplification setup run. The number displayed is based on:
    - The number of sample positions in the input labware
    - The number of control wells specified
    - The number of standards specified
    - The number of standard curve and sample replicates
    - The available space in the destination labware
4. To view the administrator-defined settings for this method, press the **Variant Information** button.

4. Instrument Setup Instructions

Below is an image and table indicating the general layout of the Maxprep™ Liquid Handler and the positions of all required and optional labware for this method.



Position	Reagent/Labware	Notes
A[1]	Dilution Plate	1.1ml Square-Well V-Bottom Deep Well Plate
B	Amplification Plate	User-specified amplification plate
C	Input Sample Positions	Samples in Maxwell® deck trays, samples in plates, or samples in tubes.
D	Reaction Mix Preparation; up to three (3) empty 1.5ml tubes as prompted	Maxprep™ 3-Position Reagent Tube Holder
E[1]	Amplification Controls; up to three (3) individual 1.5ml tubes	Maxprep™ 3-Position Reagent Tube Holder
F[1,3]	Standard (Position 1) and Standard Diluent (Position 2)	Maxprep™ 3-Position Reagent Tube Holder.
G[1]	Sample Diluent	Maxprep™ Reagent Reservoir, 50ml
H	Up to 3 tubes of Amplification Master Mix or Buffer	Maxprep™ 3-Position Reagent Tube Holder
I	Up to 3 tubes of Primer and Probe Mix	Maxprep™ 3-Position Reagent Tube Holder
J[1]	Up to 3 tubes of Enzyme	Maxprep™ 3-Position Reagent Tube Holder
K	Up to 3 tubes of Amplification-Grade Water	Maxprep™ 3-Position Reagent Tube Holder
L[1]	Labware for Standard Curve, Water Transfer or both[2]	Amplification plate base with strip tubes in column 1, 12 or both.
M	300µl Conductive Disposable Tips, Filtered	Partial or full rack
N	50µl Conductive Disposable Tips, Filtered	Partial or full rack
O	50µl Conductive Disposable Tips, Filtered	Full rack

[1]Optional labware positions based on variant settings.
[2]The standard curve labware is used to hold amplification-grade water and prepare standard curve serial dilutions.
[3]Position 1 is the rear position and Position 2 is the middle position in the Maxprep™ 3-Position Reagent Tube Holder.

5. PCR Protocol

The Maxprep™ Liquid Handler will prepare amplification reactions as indicated by the variant selected. The actual steps performed by the method will depend on the settings made in the variant method. Below are all of the steps that can be performed by the Maxprep™ Liquid Handler, depending on variant settings:

1. The system checks that there is a sufficient volume of sample diluent to dilute samples and controls.
2. Prepare amplification reaction mix in one or more 1.5ml tubes for all samples, controls and standards based on the volumes of the following components specified in the variant method:
   a. Amplification Master Mix or Buffer
   b. Primer and Probe Mix
   c. Enzyme
   d. Water in Master Mix
2. Transfer sample diluent from the sample diluent trough to the dilution plate.
3. Transfer standard diluent to column 1 of the standard curve labware.
4. Serial dilution of standards in column 1 of the standard curve labware.
5. Transfer water to column 12 of the standard curve labware.
6. Dilute samples and controls 100-fold in the dilution plate.
7. Transfer reaction mix to the amplification plate.
8. Transfer water to the amplification plate.
9. Transfer samples to the amplification plate.
10. Transfer standards, controls or both to the amplification plate.
11. Method is complete. Open the instrument door, remove the amplification plate and centrifuge at 500 × *g* for 30 seconds to remove any bubbles. Prepare the plate per your amplification protocol. Remove primary samples from the system and store them. Discard used labware as hazardous waste following your institution's recommended guidelines. Either discard or tightly cap and store remaining reagents.

Consumables for Maxprep™ methods are designed to be used with potentially infectious substances. Use appropriate protective equipment (e.g., gloves and goggles) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.

6. Variant Method Variables

Administrators should create laboratory-specific variants of the PCR method for each unique amplification plate setup they wish to create. The following sections describe the variables that can be adjusted by administrators. The only variable that can be adjusted by users at run time is the 100-fold dilution of samples. For all other variables listed, the administrator should define the default value for the variable within the displayed minimum and maximum allowable values.

Controls

These variables define the layout of controls on the amplification plate. Up to three different control types can be used within a run, and multiple well locations can be defined for each control type. Options within the table below are available for each of three control types. Within the table, X indicates the value 1, 2 or 3.

Note: Placement of controls on the plate occurs sequentially. If the same well location is specified for controls 1, 2 and 3, only control 3 will be placed at that location. If the control is placed at a well location specified for the standard curve, only the standard curve will be placed at that location.

Setting	Details
Control X Name	Name of the control; identifies the control during instrument setup.
Control #X Concentration	Concentration value of the control; used for reporting purposes.
Control #X Well Numbers[1]	Well locations where the control should be placed. For multiple control wells, specify the well locations as a comma-delimited list (e.g., 1, 17).

[1]Well numbering proceeds down columns and across the plate. For example, A1 = 1, B1 = 2, A2 = 9, etc. A well numbering diagram is displayed in the *Amplification Setup Methods for the Maxprep™ Liquid Handler Technical Manual* #TM526.

Reaction

These variables define the volumes of reaction components used to create the amplification reaction master mix. The total reaction volume is the sum of the volumes of each component and the sample. The maximum total reaction volume is 50µl per well.

Setting	Details
Buffer Volume (µl)	Volume of amplification buffer or amplification master mix per reaction.
Primer Volume (µl)	Volume of primer mix per reaction.
Enzyme Volume (µl)	Volume of amplification or reverse transcriptase enzyme per reaction.
Water Volume in Reaction Master Mix (µl)	Volume of amplification-grade water to be added to the reaction master mix per reaction.
Water Volume Transferred with Sample (µl)	Volume of amplification-grade water to transfer with the sample per reaction. This can improve transfer accuracy at low sample volume.
Sample Assay Volume (µl)	Volume of sample per reaction.

6. Variant Method Variables (continued)

Samples

These variables define how a method should process samples for the amplification setup.

Setting	Details
Dilute Samples 100-Fold	Check this box to dilute samples and controls 100-fold prior to amplification setup. Users can modify this variable at run time; toggle the "User Modifiable" check box to allow or prevent user access at run time.
Sample Replicate Number	Specify the number of replicates that should be prepared for each sample. The entire set of samples is placed on the amplification plate as a group, and then the entire group is replicated.

Standards

The standards variables define the concentration of the standard stock, the concentration of each standard, the number of standard curve points and the number of standard curve replicates to use for the amplification setup. A standard curve does not need to be prepared during reaction setup.

Note: To perform a PCR amplification without a standard curve, leave the **Prepare Standard Curve** box unchecked.

Prepare Standard Curve

This check box determines whether a standard curve will be prepared for an amplification setup. Check the box to create a standard curve as defined by the Standards variables. Leave the box unchecked to prepare amplification reactions without any standard curve, and all other variant options in **Standards** will be ignored.

Standard Concentration

Concentrations for up to eight standards can be specified to define the standard curve for the amplification plate. Concentration units are not provided for the standards to allow each laboratory to define the units (e.g., ng/µl, pg/µl, copies/µl, etc.); however, all standard concentrations must be specified in the same unit. The first standard must have the highest concentration with each subsequent standard decreasing in concentration. The final standard to be used in the standard curve should be a No-Template Control (NTC) with a concentration of 0. Any standard positions that are not to be used should be assigned a concentration of –1. Standard curves will be prepared on the right side of the amplification plate with the final NTC curve point in well H12 of a 96-well plate, and the other curve points arrayed sequentially above.

Note: The maximum dilution between standard curve points should be between 2- and 25-fold.

Stock Concentration for Standards

The concentration of the standard stock should be entered in the same unit defined for the standard curve points. The standard stock must have a concentration equal to or higher than the concentration of the first standard in the standard curve. The stock concentration must be no more than 25X of the highest concentration of the standard curve.

Standard Curve Replicates

This variable specifies the number of standard curve replicates that should be prepared on the amplification plate. Standard curves are placed on the lower right side of the amplification plate with each successive standard curve replicate shifted one column to the left. As the number of standard curve replicates increases, the total number of samples that can be processed on a given amplification plate decreases.

7. Summary of Changes

The following changes were made to the 1/24 revision of this document:

1. Added new catalog numbers to the cover page.
2. Updated font and cover image.
3. Made minor text edits.