PlateLoc Thermal Microplate Sealer

Quick Reference Guide

Agilent Technologies

Notices

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PlateLoc Thermal Microplate Sealer

Reference for Optimal Thermal Microplate Sealing

This reference contains the following topics:

Agilent Technologies

About this guide

This guide provides a quick-reference for using the PlateLoc Thermal Microplate Sealer (PlateLoc Sealer). Use the following guidelines to identify thermal-sealing-friendly labware and optimize microplate sealing using the PlateLoc Sealer. You should use this guide in conjunction with the following:

- *PlateLoc Thermal Microplate Sealer User Guide*
- *PlateLoc Seal Selection Guide*

You can download the latest version of any PDF file or search the online knowledge base from the Automation Solutions Knowledge Base page.

For more information about automation products, go to the Agilent Technologies website at www.agilent.com/lifesciences/automation.

Optimizing the sealing quality

Introduction

The PlateLoc Sealer applies a sealing material on top of microplates to seal individual wells. Sealing the wells protects the contents from evaporation, condensation, oxidation, and cross-contamination during transport or storage. The PlateLoc Sealer can be used as a standalone device or in a lab automation system. The device accepts microplates made from a variety of materials.

The ideal sealing conditions can depend on a number of factors, especially the labware. Using thermal seal-friendly microplates is key to protecting samples and critical to successful sample sealing and storage applications.

Microplate sealing tips

The seal quality depends on many factors, including microplate design, material, lot-to-lot consistency and features, the sealing parameters, whether the microplates require an insert for support, and the type of seal used.

Microplate design

Automation-friendly and thermal-seal-friendly labware ensures successful sealing.

- Use only microplates that comply with standards established by the Society of Biomolecular Sciences (SBS).
- Raised chimneys around each well produce the best results.
- Avoid microplates that have a raised rim close to the perimeter of the wells. The rim can prevent uniform contact between the hot plate and the wells.

Figure 1 illustrates these features.

Figure 1 Microplate physical features and nomenclature

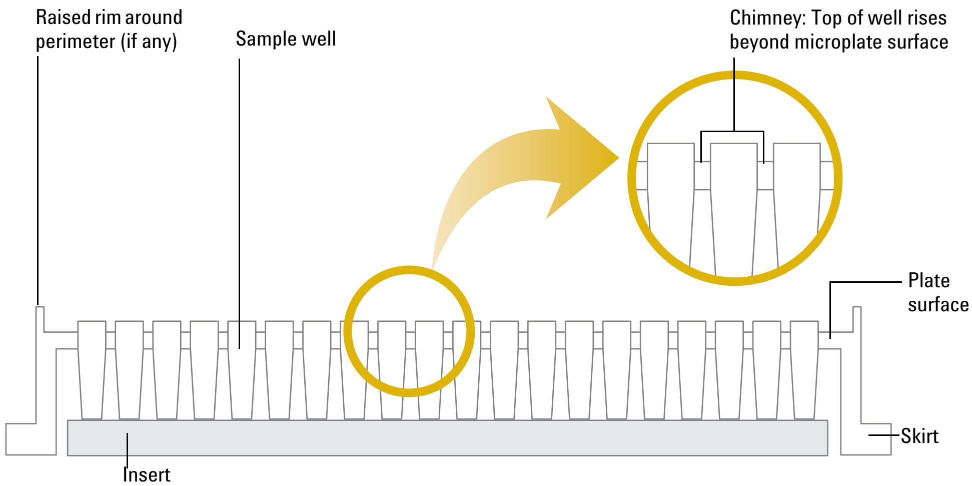
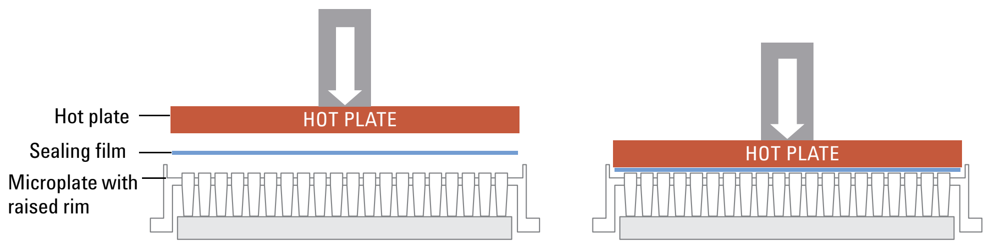


Figure 2 Left: Pneumatics drive the hot plate down to the microplate surface. The hot plate can sense and automatically adjust for variations in microplate height. Seals are applied using pressure and heat. Right: If the microplate has a raised rim that is higher than the chimney height around its perimeter, the hot plate must fit within the rimmed area so that the hot plate can make uniform contact with the seal and all sample wells simultaneously.

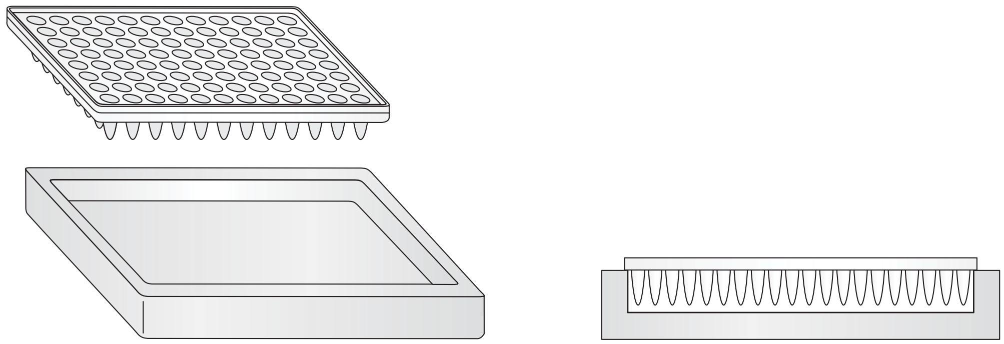


Microplate material

For the best sealing results:

- Use polypropylene microplates because they are the easiest to seal effectively and reliably.
- Avoid acrylic microplates because they are not compatible with thermal microplate sealing.
- If the microplates are flexible and tend to bend and move during the sealing process, use adapter trays to stabilize the microplate position on the plate stage. For example, place a flexible 96-well polypropylene microplate on top of a 96-well polystyrene microplate to provide positional support. Agilent Technologies also offers an adapter tray for PCR microplate support.

Figure 3 Left: Microplate and PCR Plate Support. Right: Microplate in PCR Plate Support.



Microplate make and model

- Be aware that similar microplates made from the same material but from different manufacturers may perform differently.
- Evaluate every microplate make and model to ensure consistent results.

Microplate features

Be aware of design features on the top surface of the microplate that can significantly affect the quality of the seal.

- The microplate lacks raised chimneys and it has molded branding or legends, such as the manufacturer's name or logo, or the sample locating legends (for example A, B, C, . . . and 1, 2, 3 . . .) close to the tops of the wells (close enough so that seal touches or covers the imprint).
- Tooling marks or manufacturing aids, such as circular depressions (sometimes with branching) caused by ejection pins or part of the molding process. Gaps or gutters may not only prevent binding of the seal, but may actually facilitate cross-contamination of liquid samples.

Sealing parameters

The following parameters can be adjusted to optimize the sealing results:

- Temperature
- Time

Perform optimization tests to determine the best values to use. For details, see "Optimizing the sealing parameters (temperature versus time)" on page 10.

Insert thickness

Choose the right insert to support the microplate and ensure uniform sealing. A set of four inserts are presently shipping with every PlateLoc Sealer, and a special insert is available for Labcyte microplates.

Figure 4 Microplate without and with insert

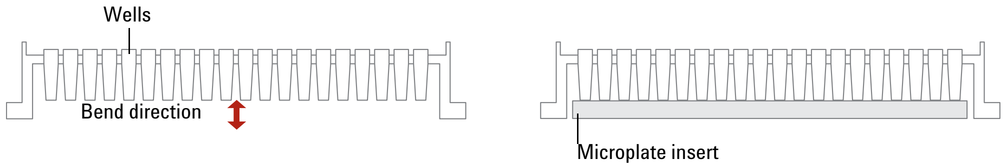
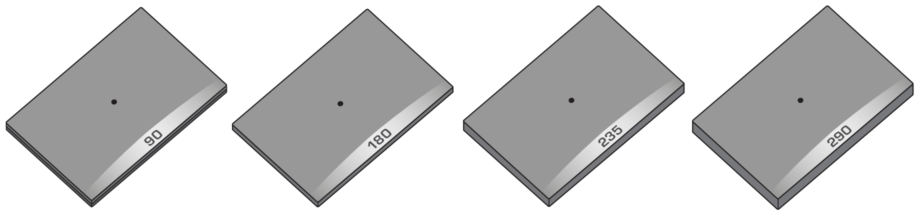


Figure 5 Standard inserts. The number on the inserts indicates the thickness of the metal pad (for example, 180 means 0.180 in thick). Note: The 90 insert has a 0.90-in metal pad with foam padding on the microplate-facing side and is used with microplates that require flexible support.



Seal material

Different seal materials have different properties and can produce different results. Perform optimization tests to determine the best seal to use.

Optimizing seal parameters

About this section

This section explains how to inspect the seal quality and, if necessary, how to adjust the sealing parameters to improve the seal. Before proceeding, download a copy of the *PlateLoc Seal Selection Guide* from the Agilent Technologies website at http://www.chem.agilent.com/Library/selectionguide/Public/5990-3659en_lo%20CMS.pdf.

Apply a seal using the time and temperature starting points suggested in the *PlateLoc Sealer Selection Guide*, for your chosen seal and microplate material. Examine the well impressions made on the seal. The pattern and quality of these impressions is a good indicator of seal quality.

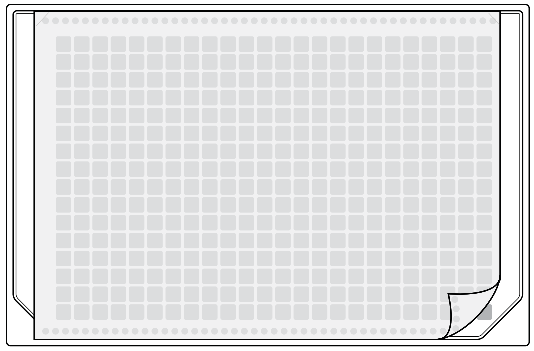
Evaluating the seal

After the seal cycle is finished, wait for the microplate to cool down enough so that it is safe to handle (10 to 30 seconds). Do not handle the microplate immediately after the seal cycle is finished. The sealed microplate might be hot.

Note: Pierceable seals might permanently adhere to the microplate. Do not wait too long or the seals will be difficult to remove.

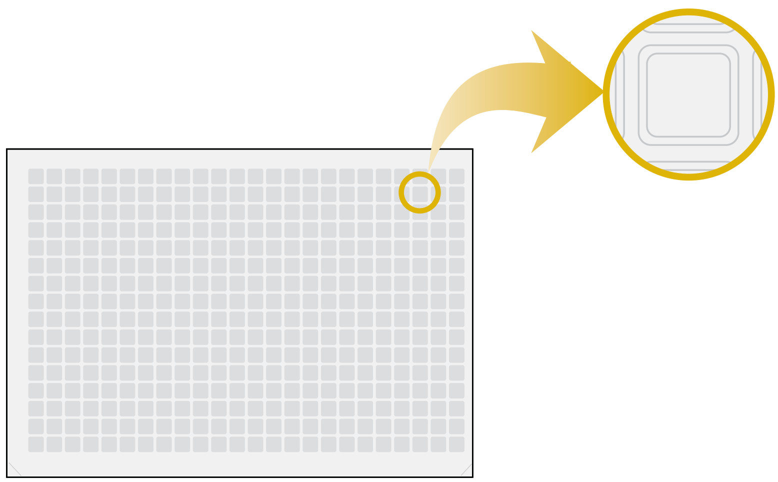
Remove the microplate from the PlateLoc Sealer. Carefully peel off the seal material by lifting one of the corners of the seal as shown in Figure 6.

Figure 6 Removing the seal.



Inspect the underside of the removed seal material. If there are unbroken impressions of each well on the underside of the seal material, the microplate was properly sealed.

Figure 7 Example of a good seal.



Use a magnifying glass or inspection microscope, if necessary, to see the area surrounding the sample well rim.

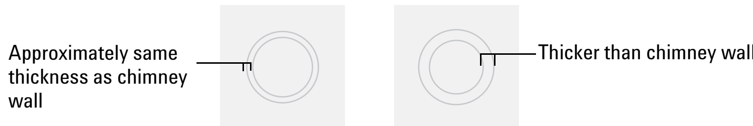
Look closely at a single sample well. A good seal might look like the illustrations on the left side of Figure 8. The well impression should match the thickness of the chimney wall, as shown in the left side of Figure 9. A poor seal might look like the illustrations on the center or right side of Figure 8, indicating there may not have been enough heat applied to form a complete impression of the sample well rim.

Figure 8 Evaluating the seals of sample wells. L: Good impression, with defined edge of chimney/rim. C and R: Poor impression, with missing or very faint segments.

If there are faint or broken impressions in the seal material, as shown in Figure 8, increase either the sealing temperature or time (duration).

If the well impressions on the seal are significantly thicker than the chimney wall (as shown in Figure 9), decrease the sealing temperature or duration. Thick impressions may indicate excessive melting of the seal, causing the seal to be difficult to remove and the resealing capacity reduced.

Figure 9 Evaluating rim width of seal. L: Good impression, with thin, defined edge of chimney/ rim. R: Poor impression, with increased width.

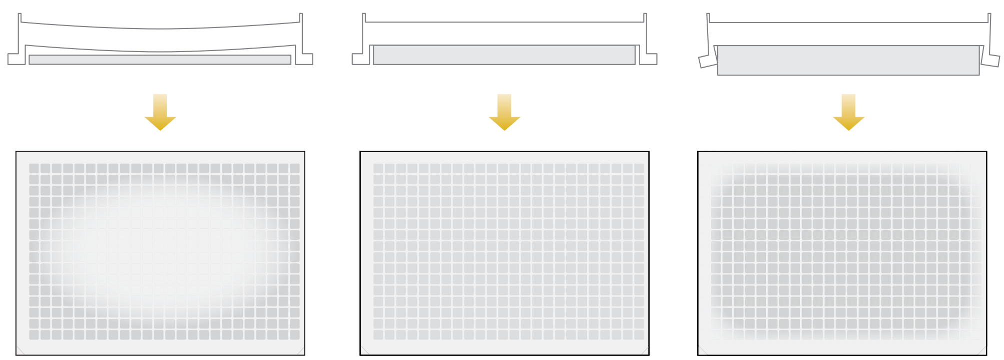


Issues related to inserts

The hot plate is a heated metal surface that presses the seal onto the microplate. For optimal sealing, the microplate needs to be firmly supported so that it does not bend during the sealing process, often requiring use of an insert to keep it flat. Figure 10 illustrates the effect of insert height on microplate sealing. Left: if the insert is too thin, the center of the microplate will bend downward during sealing, leading to good sealing around the edges, but poor sealing in the middle of the microplate. Center: if the insert is the correct thickness, the entire microplate surface will be sealed properly. Right: if the insert is too thick, the edges of the microplate will bend downward during sealing, resulting in good sealing in the middle of the microplate, but poor sealing around the edges.

Note: Thicker inserts are preferred over inserts that are too thin.

Figure 10 The effect of using inserts on microplate sealing. L: Insert too thin; C: Insert correct height; R: Insert too thick.



Requesting application support

To help expedite your support request, you can complete and submit the *Request for PlateLoc Sealer Application Support*.

For other questions, you can contact Automation Solutions Technical Support at one of the following:

Europe

Phone: +44 (0)1763853638

email: euroservice.automation@agilent.com

US and rest of world

Phone: 1.800.979.4811 (US only) or +1.408.345.8011

email: service.automation@agilent.com

Frequently asked questions

Consumables—seal

Q

Is the PlateLoc Sealer compatible with other third-party sealing materials?

A

Agilent Technologies does not verify, validate, test, or support the use of third-party sealing material on the PlateLoc Sealer. There is also some risk that non-Agilent Technologies sealing material may melt onto internal (non-user-serviceable) components, necessitating service.

Hot plate temperature verification and validation

Q

During instrument calibration, does Agilent Technologies check that the hot plate is providing the temperature indicated on the LCD screen of the PlateLoc Sealer?

A

We don't. The temperature reading is the average of two thermocouple readings, which can vary slightly from thermocouple to thermocouple. The thermocouples aren't calibrated because we have found little variation in their measurements.

If the thermocouple readings differ significantly, an error message is generated. The precision of the heating element is more important than its accuracy. Being able to precisely adjust the temperature is more crucial to optimizing sealing parameters than setting an accurate temperature. The same microplate and seal can require different sealing parameters depending on the amount and type of liquid in the microplate, the temperature of the microplate, and the desired seal strength, so absolute temperature readings are not critical to good sealing.

Hot plate cleaning

Q

I accidentally installed the thermal seal roll upside down (bonding layer up). I now have seal melted on the hot plate. Are there approved cleaning procedures? Is there a way to prevent this from happening again?

A

If Agilent Technologies installed your PlateLoc Sealer, ask for training on cleaning the hot plate. Through routine use, the hot plate will eventually become dirty from excess seal build-up, melted pieces of microplate, and general dirt and debris. The *PlateLoc Thermal Microplate Sealer User Guide*, which can be downloaded from the Agilent Technologies Automation Solutions Knowledge Base, describes routine maintenance, including cleaning the hot plate and touch screen.

To eliminate the chance of mis-loading the seal roll, use the seal-loading card that comes with every roll of Agilent Technologies seal. This card features pictures and instructions for loading new rolls of seal. Detailed information can be found in the *PlateLoc Thermal Microplate Sealer User Guide*.

Maximum sealing temperature

Q

What is the highest temperature and longest duration I can set on my PlateLoc Sealer?

A

The maximum temperature is 235 °C. The duration can be adjusted from 0.5 to 12 seconds.

Optimizing the sealing parameters (temperature versus time)

Q

I am not getting an effective seal because the sealing material is not adhering very well. Can I keep boosting the temperature or is it better to just increase the time?

A

The most common mistake new users make is to start at too high a temperature or continue increasing the temperature unnecessarily. Excessive heat can damage the microplate and prevent future resealing by prematurely melting the chimney surrounding the top of the sample wells. Refer to the *PlateLoc Seal Selection Guide* that can be downloaded from the Agilent Technologies Automation Solutions website. The selection guide recommends starting temperatures based on seal type. In general, we recommend sealing at the lowest temperature possible for the shortest time.

Resealing the same microplate

Q

We are evaluating one of Agilent Technologies peelable seals. How many times can we seal and reseal a single microplate?

A

If you minimize the temperature and time required to seal the microplate, the well chimney should usually last a minimum of five sealing cycles. Many more successful cycles may be possible, depending on the seal material, microplate material, sealing parameters, etc.

Seal cooling time

Q

We're trying to optimize sealing parameters, so we seal a microplate, wait for it to cool, then peel off the seal by hand and examine the well impressions on the back of the seal. How long should we wait before peeling the seal off?

A

We suggest waiting a minimum of 10 seconds or as long as it takes for the sealing film on the microplate to return to room temperature.

Removing seal from cold storage microplates

Q

We'd like to seal our pharmaceutical microplate library, and store the sealed microplates at −20 °C. Do we need to allow the microplates to return to room temperature prior to peeling off the seal?

A

Yes. Sealed microplates that have been stored in a refrigerator or freezer should be returned to room temperature before attempting to remove the seal. It is good laboratory practice to thoroughly test your methods prior to sealing an entire compound library. Be sure to choose a seal material that is designed for low-temperature storage and is removable (peelable). Note that some seals are designed to not be peeled off, but rather pierced mechanically for sample access.

Minimizing or eliminating cross-contamination

Q

How careful do we need to be when we manually peel off the seal by hand? What if there is liquid under the seal? Can droplets from one well contaminate another?

A

There is always the chance that condensate or liquid from a sample well may be transferred to the underside of the sealing film. One option is to centrifuge the microplate before removing the seal. Another option is to not remove the seal—use a pierceable seal. Whether you choose peelable or pierceable seal, it's best to centrifuge the microplate before removing samples.

Gas-purging option

Q

The samples we have are in DMSO and we plan to use the PlateLoc Sealer to seal our microplates with Agilent Technologies Peelable Aluminium Seal PN 06643.001 prior to putting our microplates into storage at −20 °C. How might we further reduce hydration and oxidation while sealing these microplates?

A

The Agilent Technologies gas-purging PlateLoc Sealer was developed for pharmaceutical customers to replace the air (containing moisture and oxygen) in their microplate wells with a dry, inert gas such as argon before the microplates are sealed. Because of the permeability of microplate materials such as polypropylene and polystyrene, the gas-purging effects typically last up to 24 hours when the microplates are stored at room temperature, perhaps longer if stored at lower temperatures.

Microplate-sealing technology and automation

Q

We are overwhelmed by the technology choices currently available for sealing microplates (thermal, adhesive, laser, cap mats, etc.). Is any one actually better than the other?

A

The best choice is application-dependent. To determine whether a manual, semi-automated, or automated approach would be cost-effective for your lab, evaluate how many microplates you are sealing today and how this might change over the next 12 months.

There are benefits to all sealing approaches. Each approach has strengths and weaknesses that must be weighed against the cost and benefit to your specific samples.

Thermal microplate sealing is a proven approach and is generally believed to pose the least risk of chemical incompatibility (samples and seal material) when compared to adhesive sealing. Thermal sealing subjects the top of the microplates to a few degrees of heat transfer, but the heat is typically not enough to affect most samples in life science applications. Thermal seals also typically cost less than adhesive seals.

Adhesive microplate sealing employs pressure-sensitive adhesive films (PSAs) and is most commonly used for thermally sensitive samples. Protein crystallography and some cell biology applications are instances in which customers most often use adhesive sealers.

Heat-sensitive samples

Q

We want to seal our microplates to safely store our samples, but our samples are heat sensitive. A colleague suggested we consider adhesive sealing technology. Exactly how much heat will our samples be exposed to if we use a PlateLoc Sealer?

A

Most commonly, the PlateLoc Sealer is set to 130–170 °C for a duration of 1–2 seconds. Under these conditions, the temperature of the top of the microplate, and possibly the sample, could increase a few degrees. However, this can vary based on microplate material, microplate height, sample volume, etc. Agilent Technologies recommends that you verify that the PlateLoc Sealer does not adversely affect your samples.

Continuous (24/7) operation

Q

We intend to share our PlateLoc Sealer in a core lab. Is it OK to operate it 24/7?

A

The PlateLoc Sealer is robust and designed for challenging, high-throughput, continuous-duty, production applications. We encourage you to use it for your most demanding applications. We also encourage you to talk to other PlateLoc Sealer customers, or consider an extended warranty.

Evaporation verification and validation

Q

Do you have an established protocol we can use to test the PlateLoc seal for permeability and evaporation?

A

A simple way to test for evaporation uses an experimental microplate and a control such as a 100-g weight. Add an equal amount of water to all wells. Weigh both the experimental microplate and the control. Seal the microplate. Weigh both again. Place the experimental microplate into an oven to accelerate the evaporation process (we suggest 65 °C for 3 hours or longer). Carefully remove the microplate from the oven. Let it cool down to room temperature, then weigh it again. Compare the initial and final weights of the experimental microplate. Finally, weigh the control one more time to ensure the scale is performing consistently.

Seal-peeling options and ergonomics

Q

We are presently hand-peeling seals from our microplates. We are now peeling hundreds of microplates per day, and we are concerned about potential repetitive motion injuries. What are our options?

A

Some labs choose to use pierceable seals which can be pierced using automated piercing devices such as the Agilent Technologies Microplate Seal Piercer. Another option is to use a microplate peeler device. These standalone devices can be automated, and can be integrated with robotic microplate handlers such as the Agilent Technologies BenchCel Microplate Handling Workstation.

Short sample rolls

Q

Do you have free sample rolls of seal we can test with our microplates prior to purchasing an entire roll of seal?

A

Contact Automation Solutions Technical Support for a wide variety of short, sample rolls of seal for this purpose.

Low-profile microplate support

Q

We'd like to seal Aurora® 1536 low profile microplates. Is there a special microplate support available to facilitate a good seal?

A

Yes. Contact Automation Solutions Technical Support and ask for PN 17708.001

Microplate height

Q

Can low-profile high-density microplates (for example, 1536-well microplates) or tall deep-well microplates be sealed with the PlateLoc Sealer?

A

Yes. Low-profile microplates tend to bend, so you must match the microplate with the appropriate microplate support to prevent this from happening. The sample well surface and the sample well bottoms should be rigid and parallel. Inserts (see “Insert thickness” on page 4) are designed to assist in these circumstances. The hot plate is mounted in the top of the sealing chamber and descends to the appropriate height for sealing, automatically adjusting to the microplate height. Tall labware, such as deep-well microplates as tall as 51 mm (2 in.), can be accommodated. High-volume storage and collection microplates that exceed this height are not compatible with the PlateLoc Sealer (for example, Porvair Sciences 48-well microplates of 7 and 10 mL sample volume, etc.).

How does thermal sealing work?

Q

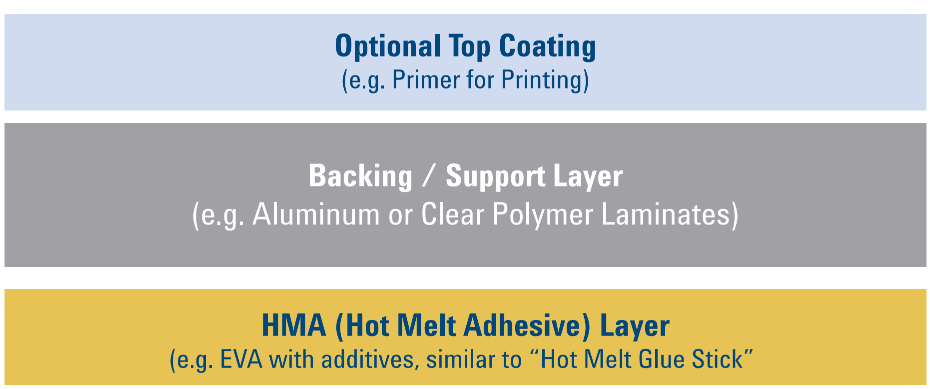
How does thermal microplate seal bond to the microplate? Will the glue contaminate my samples? Will it work with DMSO or organic solvents?

A

Hot melt adhesives (HMAs, also referred to as thermoplastic adhesives) are solid at room temperature, become molten when heated, and adhesion develops as the melt solidifies while it cools. Most hot melt adhesives achieve 50% of the bond strength after one minute, 75% after one hour, and 100% after one day.

Chemical resistance will vary based on the formulation and additives. For example, for ethylene vinyl acetate copolymer (EVA), chemical resistance varies from poor (for aromatic hydrocarbons such as benzene or halogenated hydrocarbons), to good (for oils and greases), to very good (for dilute acids, alkalis, aliphatic hydrocarbons, and alcohols). Confirm that any seal you use is compatible with your samples (see next question).

Figure 11 Hypothetical thermal microplate sealing film structure.



Chemical compatibility verification and validation

Q

We'd like to confirm the thermal microplate seal we have selected is chemically compatible with our samples for long-term storage. Do you have a suggested method?

A

Please see the Association for Laboratory Automation (ALA), Final Conference Program, January 27–31, 2001. Page 276 of the PDF contains the abstract from a poster paper generated by 3M with useful protocols for evaluating adhesive-based microplate seals.

www.labautomation.org/conference/pdfs/LA2001Book.pdf

Another useful reference is Comparison of Microplate Sealing Tapes Using Standardized Test Protocols [T-41]. Terry W. Lewis, Maurice H. Kuypers, Mialena M. Walker Medical Specialties Department 3M Health Care, St. Paul, Minnesota.

http://lab-robotics.org/Presentations/3M/3M_comparison4.PDF

Sterile and RNase-/DNase-free microplate seal

Q

Do you sell microplate seals that are certified to be sterile as well as RNase-/DNase-free?

A

No, not at this time. The majority of PlateLoc Sealer customers have been able to use the existing materials. Agilent Technologies can develop seal materials with these features, provided the customer is willing to invest the time and substantial cost required to do so.

There are also costly technical challenges to offering materials such as aluminium seals in a sterile format. Since e-beam sterilization cannot penetrate aluminum very well, gamma radiation is the only practical solution. However, the cost is substantial. Contact Agilent Technologies for more information.

Water bath thermal cycling

Q

Are Agilent Technologies thermal microplate seals compatible with water bath thermal cycling?

A

The aluminium seal can be used for a limited time in a water bath, and the clear seal for longer periods. However, the hot melt adhesive layer may be damaged by prolonged exposure to water. Also, it is very important that no air bubbles migrate into the gap between the seal surface and the top of the microplate (the gap is due to the raised rim or chimney of the sample well). For example, if two microplates are held vertically together (seal surface to seal surface) and air bubbles rise into the gap between the seal and the microplate surface itself, the expanding bubbles may apply force to the seal which may loosen the seal. One workaround is to place a piece of conforming foam between the two sealed microplates, held with sufficient force to prevent the air bubbles from entering the gap between the seal and microplate top surface.

Edge effects

Q

We are trying to seal microplates for an ELISA assay and are concerned about potential edge effects. Any suggestions on how to reduce or eliminate the problem?

A

Edge effects, such as variability in the sample data due to well-to-well differences in evaporation, can be a challenge. Rather than optimize the microplate or sealing parameters, some labs don't use the sample wells in the outer rows of the microplate, although this impacts both cost and throughput. Microplate manufacturers are incorporating new microplate design features to help reduce or eliminate the problem. Experimenting with these new designs may improve your results and throughput, providing they meet the key sealing criteria previously discussed—for example, having raised sample-well chimneys.

Application Support

Q

We have additional questions and need help with our applications. How can we request help?

A

To help expedite your support request, you can complete and submit the *Request for PlateLoc Sealer Application Support*.

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Quick Reference Guide

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