MICROPLATE SEALING

Within the Drug Discovery area of High Throughput Screening (HTS), there is an increasing need to seal microplates. It can be divided into two basic areas of use—long term and short term storage. Long term storage is typified by the library storage of compound plates. The cost of preparing the liquid store demands that its life be extended as long as feasible. The hydroscopic nature of DMSO and its affinity for water is the primary culprit in maintaining potency.

Short term storage of assay plates is being driven by the decreasing well volumes. Evaporation of the 100µL to 200µL volume of assay constituents, in a 96 well plate is of limited concern during an incubation period. However, the 10µL in a 384 well plate is a concern. As the aqueous component evaporates, the concentration of the remaining elements can alter the outcome of the assay.

The small well volumes in Genomic work creates a similar concern. This is magnified where Polymearse Chain Reaction (PCR) is used. In PCR, the well contents are not only minimal but they are heated to nearly the boiling point of water.

The reason the wells are sealed in a microplate is to preserve the contents. That also implies the need to unseal, to gain access or else there is no need to preserve. There are two basic methods of sealing microplates. Three if you count plugs, however this article is directed to applied seals. The two basic methods are the use of pressure sensitive adhesives (PSA) and heat seals.

Both of these technologies have seen marked technical advances in recent years. They are being driven by large consumer markets for lidding material. The food industry's need to seal prepared foods and then provide easy access, is a predominant demand. In the medical field, sterile packaging and its access is another large market. The food industry has a very broad range of requirements. The temperature requirements run from (-) 20°C (0°F) for frozen foods, 100°C (212°F) for boil-in-bags and higher temperatures for microwave applications. The lidding film must not only prevent evaporation but it must preserve taste and flavor. It may need to pass oxygen or inhibit its transfer.

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In the field of pressure sensitive adhesives (PSA), some very sophisticated formulations are available. Adhesives have been developed to withstand DMSO vapors. Some PSA films do not require a release liner, which simplifies their application on heat seal films. What appears to be a single thin film is actually multiple film layers. There may be a support layer and a seal layer. The seal layer itself may be a multiple layer to provide peelability. One layer bonds to the microplate. The peelability may be a function of separating the bond between the two seal layers. Fortunately the requirements for sealing microplates can draw on this field of technological developments.

To apply pressure, an air cylinder is used to close the seal platen to the plate. The bore of the air cylinder used equates to a sealing pressure at the seal surface of 4710 psi for 96 well plates and 53.6 psi for 384 well plates at 30 psi.

An extensive testing program was undertaken to evaluate heat sealing films for microplates. Heat sealing is dependent on three parameters; temperature, pressure and time. A typical 96 well plate has approximately 0.030 in^2 of sealing surface. A typical 384 well plate has 2.635 in^2. This data is based on those plates with a raised lip around the upper diameter. This is typical of most microplates on the market today. If the top surface is flat, without the raised lip, there is considerably more surface area.

A six-second seal time was chosen for the testing program. The concern with a longer time is the total heat in calories (BTU) that would be transmitted. This of course is also a function of the seal bar temperature. If an excessive amount of heat is applied then the temperature, of the contents of the wells, may be affected. The temperature of the seal bar was varied to obtain the best compromise between sealability and peelability.

The first test was to determine evaporation of water from a sealed plate, when stored for two weeks at ambient conditions. 96 well plates were filled with 200µL of Deionized water. 384 well plates were filled with 50µL of Deionized water. They were weighed daily.

The AS-1 film is a clear film. The water evaporation rate is 1.3 grams / 100in^2 / 24hours. The AS-2 and AS-3 are aluminum foil structures, containing 1¼ mil of aluminum foil. A structure with less than 1mil of foil may have pinholes, due to the manufacturing process. Aluminum foils in excess of 1mil are regarded as impermeable. The above data used an open plate and a lidded plate as controls. The average ambient temperature was 73 °F and the relative humidity was 30%. The seals were peeled after the two week test and examined. The seals of AS-1 and AS-2 on Polypropylene and Polystyrene were all rated as good. AS-3 provides a good seal on Polypropylene but is not designed to seal to Polystyrene. Some plates were of the flat surface type without the perimeter rings. These seals were rated as poor. The test results from these plates were discarded.

The next test was to evaluate the absorption of water by DMSO. Plates were filled with 150µL for 96 and 50µL for 384. To accelerate the test results they were placed in an incubator at 37°C and 90% relative humidity, for a period of two weeks. The AS-1 film failed with DMSO. The DMSO attacked the seal layer. Another aluminum foil film also failed with the DMSO test, although it gave good results on the water evaporation study.

The DMSO test was repeated with two different aluminum foil structures, AS-2 and AS-3. Following are the conclusions that were drawn from the testing program.

General Concerns

- Even when sealed with impermeable aluminum seals, there is still some vapor transfer through the walls of the microplate.
- Polypropylene plates have a lower evaporation rate than Polystyrene under the same conditions.
- Increasing the sealing pressure provides a tighter seal at the expense of peelability for access.
- Higher temperatures may deform the top of the well surface.
- Microplates with a flat top surface provide a poor seal. Those with a raised perimeter rings around the well provide a good sealing surface.
- An important requirement is to obtain a complete perimeter seal around each well. This is particularly true of the exterior wells.

Film Comparisons

AS-1 This clear film provides an excellent and easy peelable seal on both Polypropylene and Polystyrene plates. It is low cost and designed for short term storage. In two weeks there was approximately 3% evaporation. This film is not suitable for storage of DMSO.

AS-2 This is an aluminum foil seal, providing excellent seals on Polypropylene and Polystyrene plates. At ambient storage, for two weeks, of aqueous liquid there was an approximate loss by evaporation of 0.5%. At 37°C, 90% RH the increase in weight of DMSO by moisture capture was 1.5%.

AS-3 This film was comparable in performance to AS-2 on Polypropylene. It is not suitable for sealing Polystyrene plates. It has a lower seal temperature of 150°F compared to 190°F of AS-2.

AS-4 This film is a 0.5mil aluminum foil structured film for easy handling. It provides an excellent seal to polystyrene or polypropylene plates. The seal is easily peeled for ready access to the contents of the well. The cost is approximately 7¢ per seal.

Summary

Clear plastic sealing films can provide a reliable short time (2 weeks), low cost seal on both Polypropylene and Polystyrene plates. Aluminum foil material is better suited for long term storage as are Polypropylene versus Polystyrene plates. By controlling the sealing parameters of time, temperature and pressure, optimum results may be obtained equating sealing with peelability for access. A heat seal film leaves little or no reside when peeled and may be resealed multiple times.

Note:

The AS-1, AS-2, AS-3 and AS-4 material designations are for sealing films supplied by Tomtec for use on the AutoSeal. They are supplied on 3 inch diameter cores with a full roll diameter of 5 to 8 inches. All films are slit to a 3.093 inch width.