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## Eppendorf Deepwell Plates and Microplates – Investigations of sealing methods

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## Abstract

For incubation and secure storage of samples in plates it is essential that good compatibility with various sealing systems which are commonly used in the laboratory is ensured. In this Application Note, different sealings such as heat sealing, adhesive closures, sealing mats and lids are investigated in combination with the Eppendorf Deepwell Plates and Microplates. To this end, appropriate parameters for heat sealing were determined, compatibility of the closures was tested at different temperatures, and evaporation rates were analyzed. It could be shown that Eppendorf Plates may be sealed effectively using a variety of sealing options, thus providing ideal conditions for sample storage.

## Introduction

96 and 384 well plates are commonly used for laboratory procedures, as these plates simplify the handling of large numbers of samples and enable automation of certain work steps. Depending on the application, different types of plates are available which differ in volume, bottom shape or material. Since practically every experiment includes incubation steps or and/or necessitates sample storage, it is essential that plates can be securely sealed in order to avoid contamination and evaporation. Commonly used sealing systems are listed below (Table 1).

Plates made from polypropylene are advantageous for incubation steps and sample storage, as this material is very resistant to chemicals and also temperature resistant. In addition, it is possible to combine plates made from this material with different sealing options, especially heat sealing. Thus, Eppendorf Deepwell Plates and Microplates of the 96 and 384-well formats made from polypropylene provide ideal conditions for incubation and storage of sample material. Different sizes and bottom types, along with a selection of purity grades and "low binding" variants (Eppendorf LoBind) make these plates the ideal choice for a broad spectrum of applications.

In this Application Note Eppendorf Deepwell Plates and Microplates were tested for their compatibility with different sealing systems. Fit and tightness were tested and furthermore, the closures offered by Eppendorf were tested for their optimum temperature range. The sealed plates were subjected to an evaporation test at 37 °C.

## **Overview of different sealing options**

## Heat sealing: Eppendorf Heat Sealing Film and Foil

- > Films or foils are welded to the plates using a heat-sealing device.
- > Very secure and effective seal.
- > Only suitable for plates made from polypropylene or similar materials.
- > A raised rim around the individual wells is required for this type of seal.

## Adhesive sealing: Eppendorf Storage Film and Foil

(Eppendorf PCR Film and Foil display their optimal adhesion only at PCR temperatures and should not be used for storage applications).

- > Adhesive films or foils are glued the plate surface.
- > Simple handling; however, diligent and equal pressure are necessary to ensure good protection from evaporation.
- > Possible influence of the adhesive on the sample has to be taken into consideration.

## Materials and Methods

#### 1. Compatibility of sealing options

A selection of covers (heat sealing, adhesive seals, sealing mats, lids) was used in combination with Eppendorf Deepwell Plates and Microplates of different formats. The products used are listed in tables 4a and 4b in the results section. Seals were evaluated according to their fit. In addition, films and foils were tested for tightness. To this end, the plates were filled with a colored solution in a chess pattern and sealed. Following inversion and slight shaking of the plates it was determined whether cross-contamination between wells had occurred (Fig. 1).

Heat sealing devices from different manufacturers (Table 2) were used in combination with films and foils (Table 4a) to seal the Eppendorf Deepwell Plates and Microplates. The devices differ in their handling (manual to automated operation), as well as in their selection options. The uses of supports or adapters, as well as parameter selection, were performed according to the instructions of the individual manufacturers. Appropriate sealing parameters were determined to ensure a secure seal of applicable. Following plate sealing the cover was removed and its underside was examined. Even and complete

## Sealing mats: Eppendorf Sealing mats

- > Reusable mats made from an elastomer.
- > The plate can be opened and re-sealed at any time.
- > The mat needs to be compatible with the plate (round/ square wells).

## Lids: Eppendorf Plate Lid

- > Reusable transparent lid made from polystyrene.
- > Simplest and fastest closure option.
- > Mainly used as a temporary cover and for short incubations, since protection from evaporation is limited compared to other sealing options.

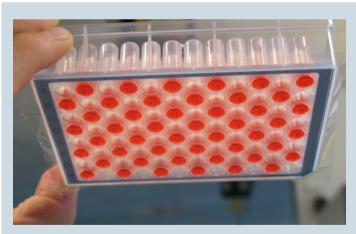


Figure 1: Sealed Eppendorf Deepwell Plate filled in a chess pattern and inverted to test for tightness.

sealing rims are a hallmark of a good and secure closure (Fig. 2a and 2b). As an example, the parameters selected for the Eppendorf HeatSealer S200 are provided in Table 3. The seals offered by Eppendorf were further tested for their temperature resistance at temperatures common in the laboratory (e.g. freezing at -86 °C, incubation at 95 °C).

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#### Table 2: Heat sealing devices tested

Device type	Device	Plate type	
Manual	HeatSealer S100 (Eppendorf)	Deepwell Plates, Microplates	
	ALPS 50 (ABgene)	Deepwell Plates	
	Xts-384 Thermal Sealer (fluidX)	Deepwell Plates	
Semi automated	EasySealer (Remp)	Deepwell Plates, Microplates	
	MiniSeal (Porvair Sciences)	Deepwell Plates, Microplates	
	HeatSealer S200 (Eppendorf)	Deepwell Plates, Microplates	
Automated	PlateLoc (Velocity11)	Deepwell Plates	
	ALPS 100 (ABgene)	Deepwell Plates, Microplates (each with their own adapter)	

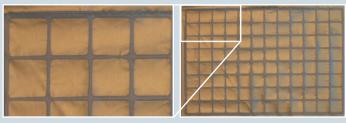


Figure 2: Foils removed from heat sealed plates a: Complete and well established sealing rims

**Table 3:** Parameters for heat sealing using the HeatSealer S200

Eppendorf Microplates					
	96/F	96/U	96/V	384/F	384/F
Heat Sealing Film	160 °C, 1.5 s	150 °C, 3 s	150 °C, 3 s	160 °C, 3 s	160 °C, 3 s
Heat Sealing Foil	165 °C, 1.5 s	165 °C, 1.5 s	165 °C, 1.5 s	175 °C, 2.5 s	175 °C, 2.5 s
Eppendorf Deepwell Plates					
	96/500 μL	96/1000 μL	96/2000 μL	384/200 μL	
Heat Sealing Film	170 °C, 1.5 s	170 °C, 2 s	170 °C, 2 s	150 °C, 3 s	
Heat Sealing Foil	170 °C, 2 s	175 °C, 3 s	175 °C, 3 s	175 °C, 3.5 s	

## 2. Evaporation rates of sealed plates

Different types of plates were filled with water according to the following outline:

> Microplate 96/V: 100  $\mu$ L

. .....

- > Microplate 384/V: 50  $\mu$ L
- > Deepwell Plate 96/2000 μL: 200 μL
- > Deepwell Plate 96/1000 μL: 100 μL
- > Deepwell Plate 384/200 μL: 100 μL

Subsequently the plates were closed with different Eppendorf closure systems and the loss of weight, and therefore the percent evaporation, was determined at a temperature of 37 °C over a period of 15 days.





**b:** Sealing is not continuous (see arrows), the sealing rims are weakly established

## Results

### 1. Compatibility of sealing options

The products listed in Table 4a may be used in combination with the Eppendorf Deepwell Plates and Eppendorf Microplates. In addition to pure seals, air-permeable closures, such as those used for bacterial and cell culture, were tested. The mats tested are listed in Table 4b. In these cases the compatibility is dependent on the size 96 wells, 384 wells) or the shape (round, square) of the wells, respectively. The temperature ranges suitable for the closures are listed in Table 5.

Table 4a: Tested sealing options (heat sealing, adhesive seals, lids)

Product	Order No. <sup>1</sup>			
Heat sealing				
Heat Sealing Film	0030 127.838			
Heat Sealing Foil	0030 127.854			
Removable Thermo-Seal <sup>2</sup>	300 21 838			
Clear Seal 37302	4ti-0580			
Clear Seal <sup>2</sup>	11452.001			
Peelable Aluminum <sup>3</sup>	06643.001			
Peelable Heat-Sealing Film2	E2796-3060			
Adhesive seals				
Storage Film	0030 127.870			
Storage Foil	0030 127.889			
Thin Seal <sup>2</sup>	100-Thin-PLT			
EZ-Pierce <sup>2</sup>	EZP-100			
Aluma Seal II	AF-100			
Seal Plate <sup>2</sup>	100-SEAL-PLT			
Sealing Tape	236366			
BREATHseal <sup>4</sup>	676050			
Gas Permeable Adhesive Seal <sup>4</sup>	AB-0718			
Aluminium StarSeal <sup>2</sup>	E2796-9793			
Lids				
Plate Lid (PCR clean)	0030 131.517			
Plate Lid (sterile)	0030 131.525			
	Heat sealingHeat Sealing FilmHeat Sealing FoilRemovable Thermo-Seal2Clear Seal 37302Clear Seal2Peelable Aluminum3Peelable Heat-Sealing Film2Adhesive sealsStorage FilmStorage FoilThin Seal2EZ-Pierce2Aluma Seal IISealing TapeBREATHseal4Gas Permeable Adhesive Seal4Aluminium StarSeal2LidsPlate Lid (PCR clean)	Heat sealing           Heat Sealing Film         0030 127.838           Heat Sealing Foil         0030 127.854           Removable Thermo-Seal <sup>2</sup> 300 21 838           Clear Seal 37302         4ti-0580           Clear Seal <sup>2</sup> 11452.001           Peelable Aluminum <sup>3</sup> 06643.001           Peelable Heat-Sealing Film2         E2796-3060           Adhesive seals         5torage Film           Storage Foil         0030 127.870           Storage Foil         0030 127.870           Storage Foil         0030 127.889           Thin Seal <sup>2</sup> E2P-100           Aluma Seal II         AF-100           Seal Plate <sup>2</sup> 100-SEAL-PLT           Sealing Tape         236366           BREATHseal <sup>4</sup> 676050           Gas Permeable Adhesive Seal <sup>4</sup> AB-0718           Aluminium StarSeal <sup>2</sup> E2796-9793           Lids         Plate Lid (PCR clean)		

<sup>1</sup> It cannot be guaranteed that the products and/or the properties of products made by a third party remain unaltered.

<sup>2</sup> The closures were only tested in combination with Eppendorf Deepwell Plates.

<sup>3</sup>The seal was tested in combination with Eppendorf Microplates by Agilent<sup>®</sup> (Agilent Sealing Guide for Eppendorf Microplates).

<sup>4</sup>Air permeable closures for cell and bacterial culture (in these cases suitability includes the successful culture of bacteria in closed plates).

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#### Table 4b: Tested sealing options (mats)

Manufacturer	Product Order No. <sup>1</sup>			
	Mats for Deepwell Plates 96/2000 μL (square wells)			
Eppendorf	Sealing mats (for Deepwell 96/2000)	0300 127.579		
Axygen	Silicon mat 96 2 ml	AM-2ml-SQ		
Greiner	CAP MAT 96 well 2 ml	381080		
Whatman	CapMats 96 well	7704-0104		
	Mats for Deepwell Plates 96/1000 μL, 96/500 μL, Microplates 96/F, 96/U, 96/V (round wells)			
Eppendorf	Sealing mats (for Deepwell Plate 96/1000)	0030 127.552		
Greiner	CAP MAT 96 well 1 ml	381061		
Micronic	TPE Capmat-96	M53000		
	TPE Capcluster-96	M53001		
	Mats for Deepwell Plate 384/200 μL, Microplate 384/F, 384/V (square wells)			
Axygen	Silicon mat 384	AM-384-DW-SQ		
Greiner	CAP MAT 384 well	384070		
Whatman	CapMats 384 well	7704-0115		

<sup>1</sup> It cannot be guaranteed that the products and/or the properties of products made by a third party remain unaltered.

Table 5: Overview of the application temperatures of the Eppendorf sealing options

Closure option	Suitable temperature range		
Storage Film	-20 °C – 120 °C		
Storage Foil	-20 °C – 120 °C		
Heat Sealing Film	-80 °C – 110 °C		
Heat Sealing Foil	-80 °C – 110 °C		
Mats	-86 °C – 120 °C		
Lids	-86 °C - 60 °C		

## 2. Evaporation rates of sealed plates

Figure 3 shows the results from the evaporation experiments for the Eppendorf Deepwell Plate 96/1000  $\mu$ L. The loss of weight, and therefore the relative evaporation, was determined over a period of 15 days. After this time, this value is clearly below 10 % for almost all closure options.

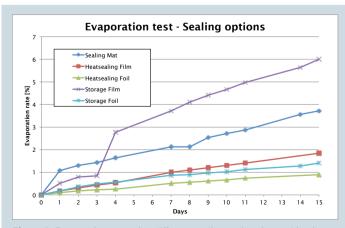


Figure 3: Evaporation test using different sealing options in combination with the Deepwell Plate 96/1000  $\mu L$  over a period of 15 days at 37 °C

## Conclusion

These results show that different closure options may be used in combination with Eppendorf Deepwell Plates and Microplates. Thus, secure incubation and storage are guaranteed for every application and temperature. The evaporation test demonstrates that almost all Eppendorf closure options offer very good protection from evaporation even after prolonged storage. The Eppendorf Plate Lid is intended for short term covering of plates. In cases where long term storage is desired, heat sealing offers the best protection. The very planar surface of the plates, which reflects consistently high manufacturing quality, contributes to their compatibility with different sealing systems. The flexible choice of closure according to the application at hand makes handling of Eppendorf Deepwell Plates and Microplates secure, simple and comfortable.

#### Ordering information Eppendorf Microplates\* (80 plates, 5 bags of 16)

Description	Quality	Well color	Border color	Order no. international	Order no. North America
Microplate 96/F-PP	PCR clean	Clear	White	0030 601.106	951040005
	Sterile			0030 602.102	951040021
Microplate 96/U-PP	PCR clean	Clear	White	0030 601.203	951040048
	Sterile			0030 602.200	951040081
Microplate 96/U-PP	PCR clean	Black	White	0030 601.807	951040102
Microplate 96/U-PP	PCR clean	White	Grey	0030 601.572	951040145
Microplate 96/V-PP	PCR clean	Clear	White	0030 601.300	951040188
	Sterile			0030 602.307	951040227
Microplate 96/V-PP	PCR clean	Black	White	0030 601.904	951040260
Microplate 96/V-PP	PCR clean	White	Grey	0030 601.670	951040308
Microplate 384/F-PP	PCR clean	Clear	White	0030 621.107	951040341
	Sterile			0030 622.103	951040383
Microplate 384/V-PP	PCR clean	Clear	White	0030 621.301	951040421
	Sterile			0030 622.308	951040464
	DNA LoBind, PCR clean			0030 623.304	951040546
	Protein LoBind, PCR clean			0030 624.300	951040589
Microplate 384/V-PP	PCR clean	Black	White	0030 621.905	951040481
Microplate 384/V-PP	PCR clean	White	Grey	0030 621.670	951040503

\*Upon request all Deepwell Plates and Microplates are available with barcode.

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#### Ordering information Deepwell Plates\* (20 plates, 5 bags of 4)

Description	Quality	Border color	Order no. international	Order no. North America
Deepwell Plate 96/2000 µL	Standard	White	0030 501.306	951033405
	Sterile		0030 502.302	951033502
	Protein LoBind		0030 504.305	0030504305
Deepwell Plate 96/1000 μL	Standard	White	0030 501.209	951032603
	Sterile		0030 502.205	951032701
	DNA LoBind		0030 503.201	951032808
	Protein LoBind		0030 504.208	951032905
Ordering information Deepwell Pl	lates* (40 plates, 5 bags of 8)			
Description	Quality	Border color	Order no. international	Order no. North America
Deepwell Plate 96/500 μL	Standard	White	0030 501.101	951031801
	Sterile		0030 502.108	951031909
	DNA LoBind		0030 503.104	951032000
	Protein LoBind		0030 504.100	951032107
Deepwell Plate 384/200 µL	Standard	White	0030 521.102	951031003
	Sterile		0030 522.109	951031101
	DNA LoBind		0030 523.105	951031208
	Protein LoBind		0030 524.101	951031305

\*Upon request all Deepwell Plates and Microplates are available with barcode.

\*\*Available in other border colors.

#### Ordering information Eppendorf HeatSealer

Description	Order no. international	Order no. North America
Eppendorf HeatSealer S100, 110-230 V	5391 000.001	5391000001
Plate Adapter, low profile, for HeatSealer S100	5391 070.018	5391070018
Plate Adapter, high profile, for HeatSealer S100	5391 070.034	5391070034
Eppendorf HeatSealer S200, 110-230 V	5392 000.005	5392000005
Plate Adapter, low profile, for HeatSealer S200	5392 070.020	5392070020
Plate Adapter, high profile for HeatSealer S200	5392 070.011	5392070011

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