forum

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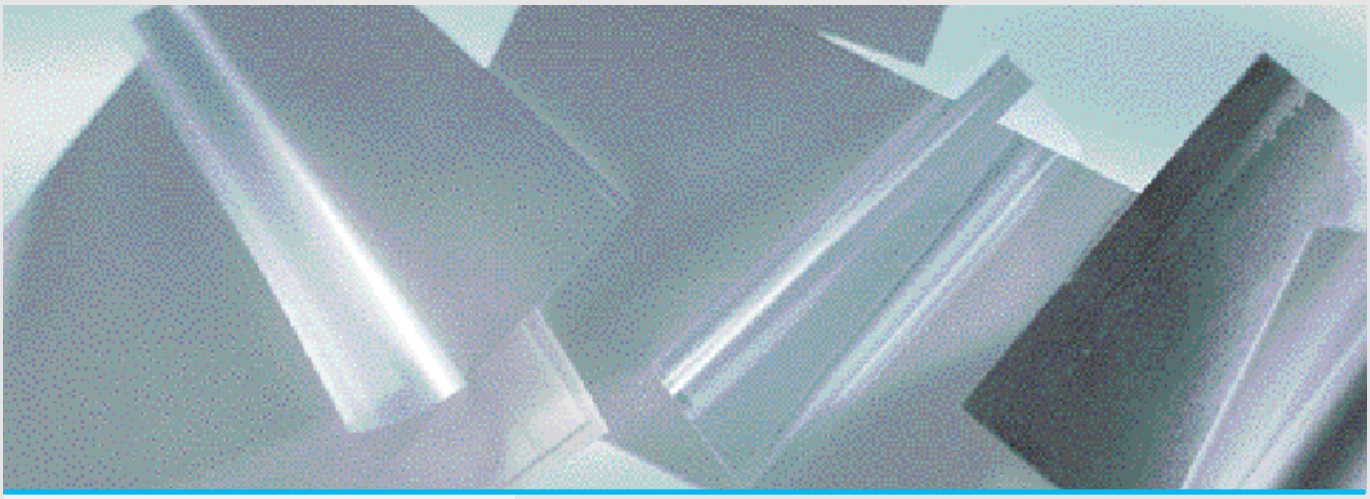
Technical Notes and Applications for Laboratory Work



Content

Sealers for microplates and their areas of application in molecular biology and cell culture

Tightly sealed microplates are a basic prerequisite for their use in molecular biology, high-throughput screening, or cell culture. At present, three different sealing methods are commonly used:

1. Heat-sealing of microplates
2. Sealing of microplates with CapMats
3. Adhesive sealers for microplates

Heat-sealing is the most widely used method, especially when sealing polypropylene storage plates for storing active substances in high-throughput screening. Heat-sealing has the disadvantage that the microplates are ultimately destroyed by the sealing process and the high temperatures may reach samples contained within the wells, especially critical for temperature-sensitive samples or cells. In addition, heat-sealing requires the acquisition of an appropriate device, which may be comparatively expensive for small research groups.

greiner bio-one

CapMats are also very popular for sealing microplates, especially in molecular biological applications such as PCR. The reuse of CapMats is widespread, but entails the risk of cross-contamination from carry-over of sample material.

In order to avoid the disadvantages of heat-sealing and CapMats, the use of adhesive sealers presents an interesting alternative.

1. Sealers available from Greiner Bio-One

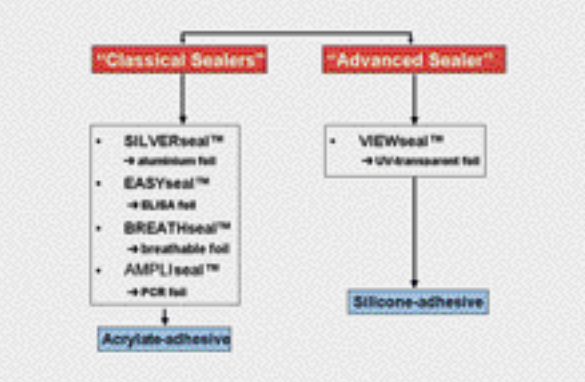


Figure 1: Classification of adhesive sealers

Greiner Bio-One supplies a full set of five different sealers that can be divided into two different classes on the basis of the adhesive used (Fig. 1). "Classical Sealers" such as SILVERseal™, EASYseal™, BREATHseal™ and AMPLIseal™ are coated with an acrylate adhesive. The "Advanced Sealer" VIEWseal™ is coated with a pressure-sensitive silicone adhesive.

EASYseal™ is the adhesive sealer for everyday laboratory coverage and protection requirements of microplates (e.g. in ELISA applications). Additionally, EASYseal™ is suitable for optical measurements in the visible wavelength range.

SILVERseal™ is a pierceable aluminium foil ideal for PCR applications and for sample storage. Thermally resistant to both heat (+110°C) and cold (-80°C), SILVERseal™ sealers remain flat when removed from their paper backing and are thus especially easily applied to all kinds of microplates.

AMPLIseal™ is a highly transparent sealer that demonstrates minimal autofluorescence, rendering it ideal for Real Time PCR. The strongly adhering acrylate adhesive provides reliable sealing without interference with PCR or fluorescence measurements.

VIEWseal™ is a highly transparent sealer ideally suited for precise optical measurements and protein crystallisation. Characterised by an exceptionally high optical transparency even in the shorter wavelength range (<340 nm), VIEWseal™ also displays minimal autofluorescence. The silicone adhesive sticks only when pressure is applied to the film surface. Thus VIEWseal™ is easy to work with, even when wearing gloves (the sealer does not adhere to them). Substances in powder form and biological model organisms like *Drosophila melanogaster* or *Caenorhabditis elegans* also do not stick to vessels sealed with VIEWseal™.

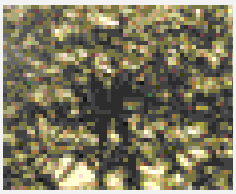


Figure 2: Microscopic image of BREATHseal™ (10-fold magnification)

BREATHseal™ is a gas-permeable, pierceable foil ideal for cultivating bacteria, yeast or cells in microplates. BREATHseal™ consists of heat-sealed rayon fibres with a pore size from 10 to 50 µm (Fig. 2). The pores are layered in such a way that the membrane acts as a filter, ensuring both reliable protection and optimal oxygen supply.

All sealers are free of DNase, RNase, human DNA and are non-pyrogenic.

2. Technical Features and Applications

Evaporation rates

Sealing properties are crucial for the usage of sealers as vapour diffusion barrier. The evaporation rate of water at 60°C in polypropylene microplates covered with adhesive sealers is shown in Fig. 3. After 20 h incubation at 60°C the evaporation rate in microplates sealed with AMPLIseal™ and VIEWseal™ was less than 2%, whereas sealing with EASYseal™ and SILVERseal™ resulted in approximately 5.5% and 7.5% evaporation, respectively.

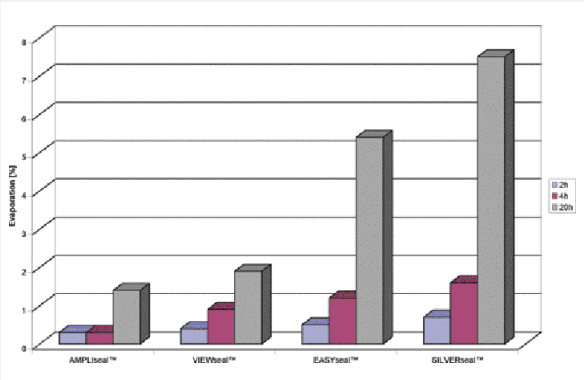


Figure 3: Evaporation rate in 96 well polypropylene microplates filled with water and incubated at 60°C for 2h, 4h and 20h, respectively.

For long-term storage of volatile substances it should be taken into account that sealers cannot provide a 100% vapour diffusion barrier. After storage of polypropylene microplates filled with isopropanol over an eight week period at room temperature, the evaporation rate for microplates sealed with SILVERseal™ and VIEWseal™ was approximately 10% (Fig. 4), and AMPLIseal™ approximately 30%.

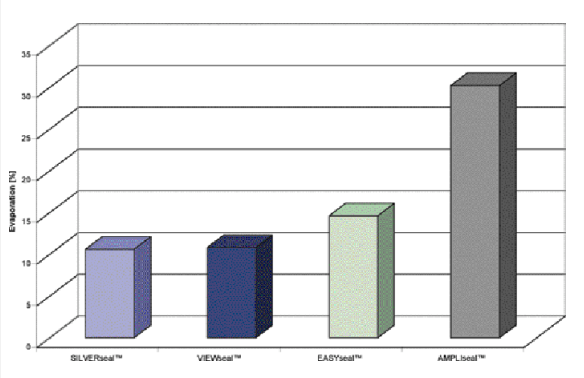


Figure 4: Evaporation rate in 96 well polypropylene microplates filled with isopropanol and stored for eight weeks at room temperature.

Optical properties

VIEWseal™ and AMPLIseal™ feature a high optical transparency, even in the shorter wavelength range (Fig. 5), as well as a low level of autofluorescence (Fig. 6). Therefore both sealers are ideally qualified for applications with sensitive optical detection systems, such as Real Time PCR or fluorescence measurements in high-throughput screening. With exceptionally low adsorption in the lower UV, VIEWseal™ is the best selection for DNA or protein measurements.

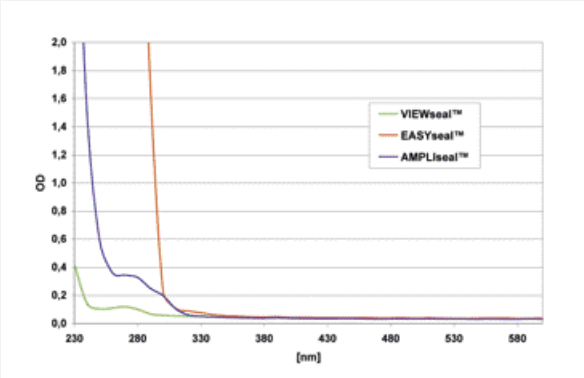


Figure 5: Light adsorption of VIEWseal™, EASYseal™ and AMPLIseal™

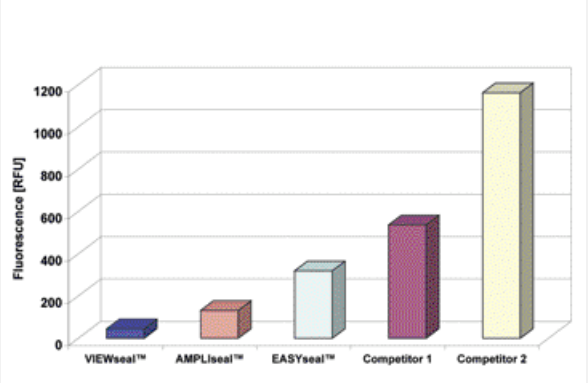


Figure 6: Autofluorescence of AMPLIseal™ compared to VIEWseal™ and other sealing films. The wavelength combination used (479 nm / 520 nm) corresponds to the excitation and emission maximum of SybrGreen

The superior optical and sealing properties of VIEWseal™ and AMPLIseal™ render them especially useful for protein crystallisation applications as well. Because the sealers are easily removed, crystals can be properly harvested without disturbing contents within the microplate wells.

AMPLIseal™ is characterised by a very low polarisation of light and therefore ideally suited for crystal detection with polarised light (Fig. 7). However, AMPLIseal™ is not resistant to some substances used for crystallisation screens, e.g. dioxane.

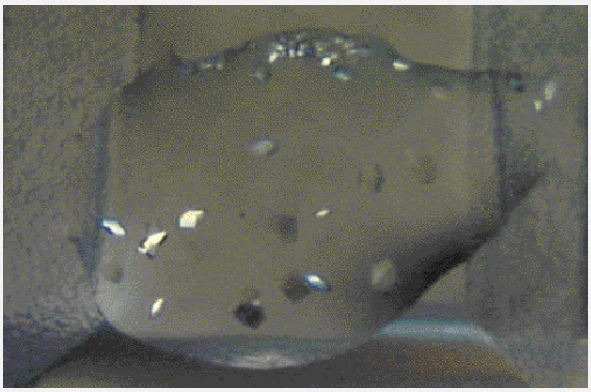


Figure 7: Microscopic detection of protein crystals with polarised light through AMPLIseal™

Chemical and temperature stability

The chemical stability of sealers is a major quality criterion for many areas of application. For testing the chemical stability of the sealers, polypropylene microplates were filled with twelve different solvents, properly sealed and then turned upside-down. As a result the sealers were in direct contact with the solvents. After one week, the sealers were inspected visually (Appendix, Tab. 2). VIEWseal™ and SILVERseal™ are clearly superior to AMPLIseal™ and EASYseal™ with regard to chemical resistance. Due to its porous nature, BREATHseal™ was not included in this test.

Temperature stability is especially important for PCR applications and storage at low temperatures. SILVERseal™, VIEWseal™ and AMPLIseal™ tolerate a wide temperature range (-80°C to 110°C) and are therefore appropriate for both PCR and sample storage at low temperatures (Appendix, Tab. 3). No significant differences were found with regard to yield and fragment sizes when sealed polyproplylene microplates were compared to PCR products from classical thin wall vessels (Fig. 8).

For EASYseal™ -20°C to 60°C is the recommended temperature range. BREATHseal™ can be used from -80°C to 60°C; however, due to its porous structure increased evaporation must be taken into account, especially at higher temperatures.

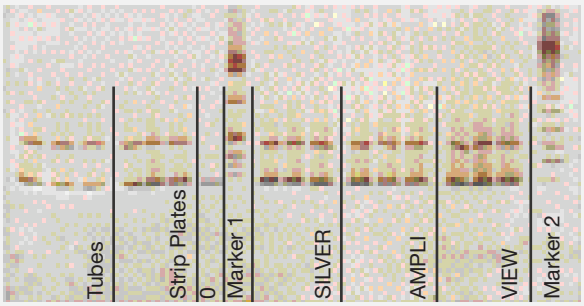


Figure 8: Comparison of PCR products from classical reaction vessels (tubes/strips) with PCR products from 96 well polypropylene microplates sealed with sealers

Cultivation of microoganisms in microplates

In many research projects, bacteria, yeast or cells are cultivated in individual vessels such as test tubes, Erlenmeyer flasks or 50 ml centrifuge tubes. With increasing automation and an increasing number of samples to be processed, 96 well MASTERBLOCK® and even 384 well microplates came into focus. Essential for optimal growth, oxygen supply is limited in microplates closed with standard sealers or lids. As a gas-permeable sealer, BREATHseal™ ensures optimal oxygen supply to significantly improve cell growth (Fig. 9) and, at the same time, provide reliable protection against contaminations. BREATHseal™ is additionally well-suited for the use of microplate shakers.

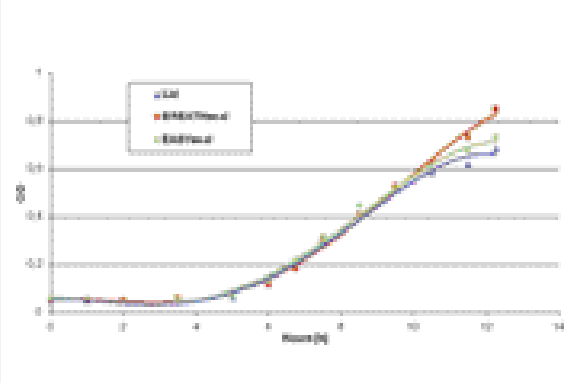


Figure 9: Growth of *Escherichia coli* at 37°C in MASTERBLOCK® sealed with BREATHseal™, EASYseal™ and lids

3. APPENDIX

Table 1: Applications

Application	SILVERseal™	EASYseal™	AMPLIseal™	VIEWseal™	BREATHseal™
PCR	yes		yes	yes	
Real Time PCR			yes	yes	
Storage	-80°C to +110°C	-20°C to +60°C	-80°C to +110°C	-80°C to +110°C	-80°C to +60°C
Cultivation				yes[1)]	yes[2)]
Optical measurements		yes[3)]	yes[4)]	yes[5)]	
Protein crystallisation			yes[6)]	yes[7)]	

1) Non-sticky
2) Gas-permeable
3) Standard optical measurements in the visible wavelength range
4) Highly transparent, for precise optical measurements, fluorescence measurements
5) Highly transparent, for precise optical measurements, fluorescence measurements, measurements in the lower UV
6) Not resistant to some substances used for crystallisation screens, e.g. dioxane
7) Slightly birefringent

This table can only be used as an orientation aid, since the behaviour of the product depends on the respective application. Tests under practical conditions are absolutely essential in many cases.

Table 2: Chemical stability of the sealers

Chemicals	EASYseal™ (Cat.-No. 676 001)	VIEWseal™ (Cat.-No. 676 070)	AMPLIseal™ (Cat.-No. 676 040)	SILVERseal™ (Cat.-No. 676 090)
Acetone	4	4	4	3
Acetonitrile	3	3	4	1
Acetic acid 1%	1	1	4	3
Glacial acetic acid	1	3	4	3
Chloroform	4	4	4	4
DMSO	3	3	3	1
Ethanol	3	1	1	1
H_2SO_4 0.5 M	1	1	1	1
HCl 32%	3	1	3	4
Isopropanol	3	1	1	1
Methanol	3	1	4	1
Phenol	3	3	4	3

1 = *Stable* = no visible change in the sealer after one week's incubation.

3 = *Moderately stable* = after one week, optical and physical changes in the sealer (clouding tears on removal).

4 = *Unstable* = adhesive and foil are dissolved, wells not leak-tight

This table can only be used as an orientation aid for the suitabiliy of the respective sealers, since their behaviour against chemicals depends on the respective application. Tests under practical conditions are absolutely essential in many cases.

Table 3: Temperature stability

Product	Temperature stability
EASYseal™	-20°C to +60°C
VIEWseal™	-80°C to +110°C
AMPLIseal™	-80°C to +110°C
SILVERseal™	-80°C to +110°C
BREATHseal™	-80°C to +60°C Evaporation rate 4200g H_2O/m^2 in 24 h

This table can basically be used as an orientation aid for the temperature suitability of the respective sealers, since the behaviour of the product depends on the respective application. Tests under practical conditions are absolutely essential in many cases.

Ordering Information

Cat.-No.	Description	Qty. per bag/case
676 001	EASYseal™, transparent	100
676 070	VIEWseal™, transparent	100
676 040	AMPLIseal™, transparent	100
676 090	SILVERseal™, made from aluminium	100
676 050	BREATHseal™, gas-permeable	50
676 051	BREATHseal™, gas-permeable, sterile	50

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Germany (Main office): Greiner Bio-One GmbH · (+49) 7022 948-0 · info@de.gbo.com, Belgium: Greiner Bio-One N.V. · (+32) 2 461 09 10 · info@be.gbo.com,
Brazil: Greiner Bio-One Brazil · (+55) 1934 68 9600 · office@br.gbo.com, China: Greiner Bio-One GmbH · (+86) 21 6272 7058 2051 · info@cn.gbo.com,
France: Greiner Bio-One SAS · (+33) 169 86 25 50 · infos@fr.gbo.com, Japan: Greiner Bio-One Co. Ltd. · (+81) 3 3505 8875 · info@jp.gbo.com,
Netherlands: Greiner Bio-One B.V. · (+31) 172 42 09 00 · info@nl.gbo.com, UK: Greiner Bio-One Ltd. · (+44) 1453 82 52 55 · info@uk.gbo.com,
USA: Greiner Bio-One North America Inc. · (+1) 704 261 78 00 · info@us.gbo.com

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