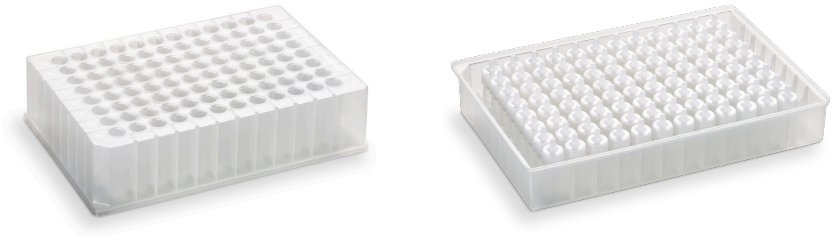
Technical Overview

# Agilent Polypropylene Filter Microplates

## Support critical analysis with excellent protein recovery and chemical compatibility



## Introduction

This technical overview demonstrates the low-binding attributes of the Agilent polypropylene filter microplates by testing the recovery of a set of six common proteins of varying sizes and hydrophobicities.

Polypropylene membranes are hydrophobic and exhibit a wide range of chemical compatibility with organic solvents (excluding esters). In addition to being highly solvent resistant, these membranes exhibit low nonspecific absorbance characteristics, resulting in maximum protein recovery for critical analysis.

These features make the membranes well suited for biological sample filtration. They are a viable choice when using acids or bases in processing, such as the processing of dry blood or other biological sample lysates. Polypropylene membranes are also an excellent choice for the filtration of HPLC samples when performing protein analysis by chromatography.

Agilent polypropylene filter plates are provided in 0.45 µm pore size in a 96-well format with well volumes ranging from 300 µL to 2 mL and long and short drip lengths (Table 1). Corresponding Agilent storage and collection plates are available for pairing. The polypropylene filter can be autoclaved and can also withstand sterilization by gamma irradiation. They are manufactured in a DNase/RNase-free and ISO 9001:2015-certified facility.

**Table 1.** Agilent polypropylene filter plates and recommended receiver plates.

| Filter Plate Part Number | Number of Wells | Pore Size (µm) | Well Volume (mL) | Drip Length Type | Recommended Receiver Plate Part Number |
|---|---|---|---|---|---|
| 201009-100 | 96 | 0.45 | 2.0 | Long | 201240-100 (204353-100 irradiated) |
| 200933-100 | | | 0.8 | Long | 201276-100 (204355-100 irradiated) |
| 200961-100 | | | 0.8 | Short | 201276-100 (204355-100 irradiated) |
| 200945-100 | | | 0.4 | Long | 204600-100 (204601-100 irradiated) |
| 200973-100 | | | 0.4 | Short | 204600-100 (204601-100 irradiated) |
| 200983-100 | | | 0.3 | Long | 203942-100 (204602-100 irradiated) |
| 200997-100 | | | 0.3 | Short | 203942-100 (204602-100 irradiated) |

## Materials and methods

### Vacuum and centrifugation testing

Agilent polypropylene filter microplates were evaluated in the 0.8 and 2 mL well sizes (Table 3). The protein solutions tested were bovine serum albumin (BSA, Sigma, part number A2153), cytochrome C (Sigma, part number C2506), gamma globulin (Sigma, part number G7516), myoglobin (Sigma, part number M1882), ovalbumin (Sigma, part number A5503), and thyroglobulin (Sigma, part number T1001). The solutions were prepared in Dulbecco's phosphate-buffered saline (DPBS, Thermo Fisher Scientific, part number 14190144) at 0.5 mg/mL. Dry filter plate weights were recorded, and filter plates were matched with appropriate Agilent collection plates (see Table 1). The filter plates were loaded with 0.2 or 0.4 mL per well (for 0.8 and 2 mL wells, Agilent part numbers 200933-100 and 201009-100, respectively) of 0.5 mg/mL proteins in DPBS or of DPBS alone (control). The samples were filtered either under vacuum at 15 in. Hg or by centrifugation at 1,500 × g to completion of filtration. When the entire plate was not used, plate seal tape was used to cover any empty wells to facilitate vacuum filtration. The weight of the filter plate was used to determine the residual liquid or holdup volume of the filter membrane after processing.

### Protein recovery testing

The protein concentrations recovered were determined using the Coomassie Plus (Bradford) Assay Reagent (Thermo Fisher Scientific, part number 23238), following the reagent vendor's protocol. The absorbance was measured at a wavelength of 595 nm using an Agilent BioTek Synergy H1 multimode reader (part number SH1M2FG). The protein concentrations were determined relative to a protein standard curve. The total protein recovered was determined using the recovered volume multiplied by the concentration and expressed as a percentage of the initial mass of protein loaded in each well.

## Results and discussion

This study was designed to evaluate the recovery of proteins in a representative protein set. Protein recovery after filtration was evaluated using a set of common proteins of varying molecular weight and hydrophobicities (Table 2).

**Table 2.** Tested proteins.

| Protein | Molecular Weight (kDa) |
|---|---|
| Bovine Serum Albumin | 66.5 |
| Cytochrome C | 12 |
| Gamma Globulin | 155 |
| Myoglobin | 17.2 |
| Ovalbumin | 45 |
| Thyroglobulin | 660 |

The protein recovery ranged from 85 to 98% and averaged 93% of the initial stock across all proteins evaluated (Figure 1). The holdup volume ranged from 1.5 to 13.9 µL per well and all processing reached completion within 5 minutes (Table 3). In general, the time required for filtration will vary depending on several factors, including the volume, solvent, and concentration of the filtered substance.

Table 4 is also included to provide guidance from the filter manufacturer and indicates the ability of a polypropylene filter to withstand exposure to a solvent. The chemical compatibility of the filter plates should be evaluated in researchers' specific application workflows.

**Table 3.** Filter plates evaluated using centrifugation and vacuum filtration.

| Part Number | Number of Wells | Pore Size (µm) | Well Volume (mL) | Volume Evaluated (mL) | Centrifugation | | | Vacuum | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Centrifugal Force (× g) | Centrifugal Time (min) | Holdup Volume (µL per well) | Vacuum Filtration (in. Hg) | Vacuum Time (min) | Holdup Volume (µL per well) |
| 201009-100 | 96 | 0.45 | 2.0 | 0.4 | 1,500 | 5 | 3.3 | 15 | 3 | 13.9 |
| 200933-100 | 96 | 0.45 | 0.8 | 0.2 | 1,500 | 2 | 1.5 | 15 | 1 | 3.9 |

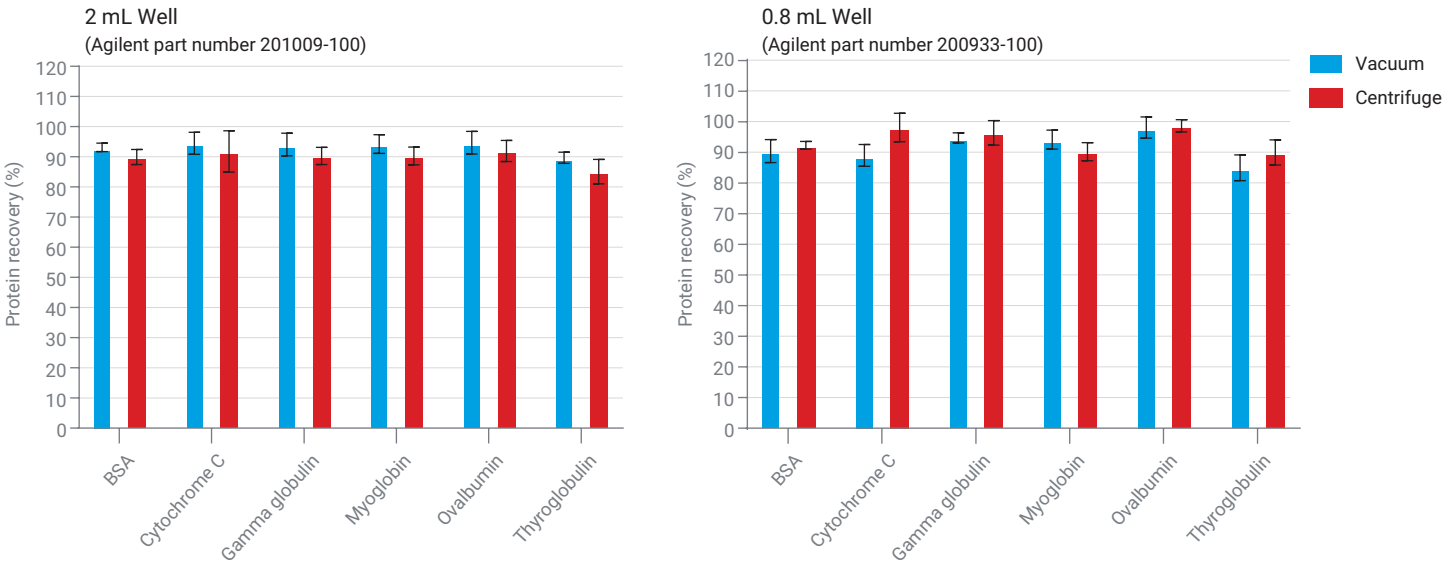



**Figure 1.** Comparison of the recovery of common proteins with a range of molecular weights using Agilent polypropylene filter plates. Error bars represent the standard deviation across three experiments.

**Table 4.** Guidance on chemical compatibility of polypropylene membrane used in Agilent polypropylene filter plates. (All concentrations 100% unless noted).

| Acids | |
|---|---|
| Acetic | C |
| Hydrochloric | C |
| Sulfuric | C |
| Nitric | C |
| Phosphoric, 25% | C |
| Formic, 25% | C |
| Trichloroacetic, 10% | C |
| **Organic Oxides** | |
| Ethyl Ether | LC |
| Dioxane/Tetrahydrofuran | C |
| Triethanolamine | ND |
| Dimethyl Sulfoxide (DMSO) | C |
| Isopropyl Ether | C |

| Amines | |
|---|---|
| Dimethylformamide | C |
| Diethyl Acetamide | ND |
| Triethanolamine | ND |
| Aniline | ND |
| Pyridine | IC |
| Acetonitrile | C |
| **Ketones** | |
| Acetone | C |
| Cyclohexanone | C |
| Methyl Ethyl Ketone | C |
| Methyl Isobutyl Ketone | LC |
| **Bases** | |
| Ammonium Hydroxide, 25% | C |
| Sodium Hydroxide, 3 N | C |

| Alcohols | |
|---|---|
| Amyl Alcohol (Butanol) | C |
| Benzyl Alcohol | C |
| Butyl Alcohol | C |
| Ethanol, 98% | C |
| Ethylene Glycol | C |
| Glycerin (Glycerol) | C |
| Isopropanol | C |
| Methanol, 98% | C |
| N-Propanol | C |
| Propylene Glycol | C |
| **Hydrocarbons** | |
| Hexane, Xylene | IC |
| Toluene, Benzene | IC |
| Kerosene, Gasoline | LC |
| Tetralin, Dekalin | ND |

| Miscellaneous | |
|---|---|
| Phenol, Aqueous, 10% | C |
| Formaldehyde, Aqueous, 30% | C |
| Hydrogen Peroxide, 30% | ND |
| Silicone Oil, Mineral Oil | C |

Legend: C = compatible, LC = limited compatibility (membrane may swell and shrink), IC = incompatible (not recommended), ND = no compatibility data currently available.

## Conclusion

Agilent polypropylene filter plates provide excellent protein recovery. This performance was demonstrated using a set of six solutions of proteins of varying sizes. The polypropylene filter membranes have a low holdup volume, which limits the loss of samples in processing. The filter material exhibits a wide range of chemical compatibility (Table 4) and is well suited for biological sample processing and for HPLC samples when performing protein analysis by chromatography. The polypropylene filter plates are compatible with vacuum and centrifugation and may also be compatible with positive-pressure devices. Processing time will vary depending on the concentration and volume of filtrate, although in this study, all proteins were filtered within minutes.

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