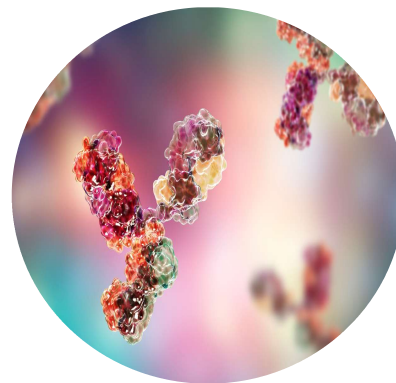


Use of Verex™ Filter Vials for Monoclonal Antibody (mAb) Fragments

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Overview

Samples are prepared in a multitude of ways, all of which can impact chromatographic results by affecting the sample injected into the system. Ineffective sample preparation can lead to reduction in column life, reduction in chromatographic separation efficiency and sample reproducibility. Choosing the wrong sample preparation device could release extractable compounds into the sample that could bind analytes of interest and influence the quality of the results observed.

For monoclonal antibody (mAb) analysis by reversed phase liquid chromatography (RPLC), common approaches are digestion with a site-specific cysteine protease such as IdeZ. In this application, an IgG1 (Trastuzumab) and an IgG4 (Nivolumab) are digested with IdeZ protease, yielding Fab2' and scFc fragments. Digested sample was then filtered using Verex Filter Vials, with either Regenerated Cellulose (RC) or Polyethersulfone (PES) membranes. Samples were then run by RPLC to determine if there was a loss of sample. When compared to mAb without a filtration device, profiles are the same, indicating no adsorption to the membrane nor vial. The use of Verex Filter Vials would be useful when working with limited sample volume, as some loss may be observed with other filtration devices.

HPLC Conditions

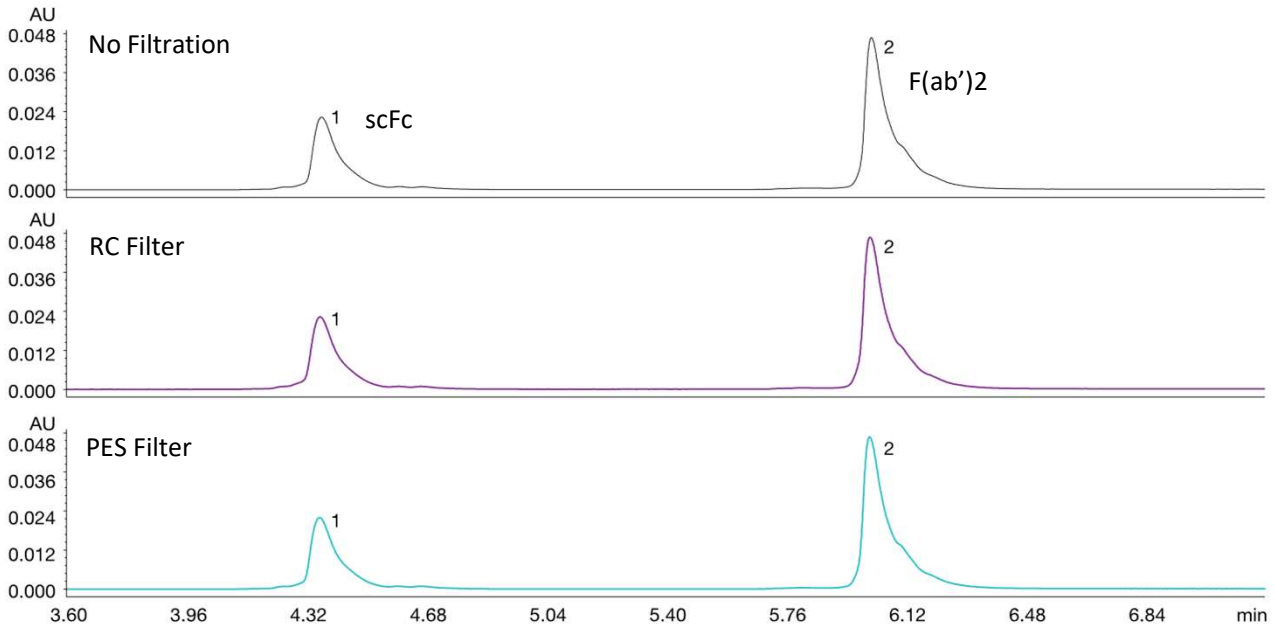
Column:	bioZen™ 2.6 µm WidePore C4	
Dimension:	100 x 2.1 mm	
Part No.:	OOD-4786-AN	
Injection:	2 µL	
Mobile Phase A:	0.1 % TFA in Water	
Mobile Phase B:	0.1 % TFA in Acetonitrile	
Gradient:	Time (min)	%B
	0	25
	10	45
	11	80
	13	80
	13.1	25
	17.1	25
Flow Rate:	0.8 mL/min	
Detector:	UV-Vis @ 280 nm	
Sample:	Nivolumab, IdeZ Digested Trastuzumab, IdeZ Digested	

Sample Preparation

Reconstitute:	FabRICATOR® Z in 50 µL ddH ₂ O to a concentration of 40 units/µL
Add:	1 unit FabRICATOR Z / 1 µg IgG. Final Concentration should be 0.5-10 mg/mL
Digest:	Incubate mixture for 16 hrs at 37 °C
Load:	Sample into Verex Filter Vial 0.45 µm, RC ARO-F203-12 0.45 µm, PES ARO-F208-12
Inject:	2 µL final eluate onto HPLC-UV

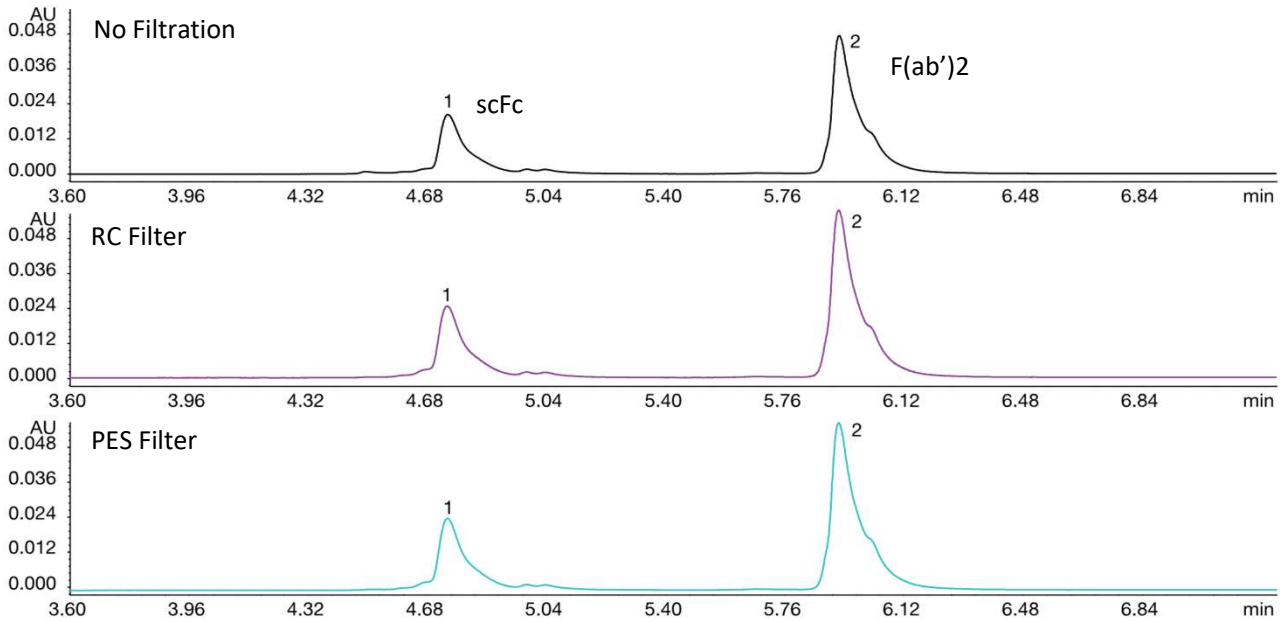


Figure 1. Trastuzumab



App ID 26412

Figure 2. Nivolumab



App ID 26413



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