BECO Validation Guide

BECOPAD P

BEGEROW

Table of Contents

1. Introduction

Depth filtration is regularly used for the separation of cells, cell fragments, colloids, precipitates, and other fermentation process components. Also, this technology is trend-setting within the filtration of media containing particles or for removal of bioburden. A further cost-effective application is the protection of final sterile filters from premature blocking using sterilizing depth filter sheets.

Clarifying depth filtration is a very important process step in the production of biological products, as it has a direct influence on the yield, product characteristics and reproducibility of the final product. Depth filtration therefore contributes to the economic efficiency and safety of the overall process.

We are committed to providing consistently high product quality in order to meet the quality requirements of our customers. As part of the manufacturing process, the depth filter medium helps to ensure safe, reproducible, and economical results for our customers.

We meet this responsibility by ensuring that our products comply with national and international quality standards such as the requirements of recommendation XXXVI/1 regarding the LFGB (German Food, Commodity and Feed Act) by the BfR (Federal Institute of Risk Assessment), and the test criteria of FDA directive CFR 21 § 177.2260 and the hygiene guidelines according to HACCP. As early as 1993, BEGEROW was certified according to DIN EN ISO 9001 and has been complying with DIN EN ISO 9001:2000 since 2002. Moreover, we make ourselves available for regular audits through external, independent institutes, and customers.

This **BECOPAD P** Validation Guide summarizes the extensive testing and evaluation program prepared by BEGEROW to meet the critical demands of the Pharmaceutical Industry.

Validation is defined as “Establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality Attributes“ (Guideline For Industry: Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice (US Department of Health and Human Services, Food and Drug Administration, September 2004).

This report contains validation data applicable to BEGEROW **BECOPAD P** range depth filter media which is used in different formats and configurations such as:

- BECO Flat Sheets for use in filter presses
- BECODISC Stacked Disc Cartridges used in stacked disc cartridge housings
- Disposable BECO MiniCaps and BECO Capsules

This validation guide has been compiled for the users of BEGEROW **BECOPAD P** range depth filter media as a basis and support for their own validation procedures.

2. General Characteristics of BECOPAD P Range Depth Filter Media

2.1 Overview of BECOPAD P types

The high-purity **BECOPAD P** range is a mineral-free depth filter medium produced to date with special, high-purity cellulose using the bepure process.

This validation report describes the following depth filter media grades, listed with increasing permeability:

BECOPAD P
BECOPAD P 120
BECOPAD P 170
BECOPAD P 220
BECOPAD P 270
BECOPAD P 350
BECOPAD P 450
BECOPAD P 550

All of these depth filter media grades are manufactured under special production conditions that guarantee the highest purity possible.

Through the use of special production methods, **BECOPAD P** depth filter media can be distinguished by very low release of extractables. Furthermore **BECOPAD P** ranging from **BECOPAD P 120** through **BECOPAD P 550** have been optimized for low endotoxin levels.

2. General Characteristics of BECOPAD P Range Depth Filter Media

2.2 Filtration mechanisms

General filtration mechanisms:
The depth filter medium offers mechanical and adsorptive mechanisms. Particles are mechanically retained in the asymmetric cavity structure of the depth filter medium (narrowing pore structure towards the filtrate side). Particles that are significantly smaller than the pores of the depth filter medium are bound through adsorption. This is due to the positive zeta potential (electrokinetic potential), which retains negatively charged particles through adsorption.

Filtration mechanisms of BECOPAD P:
In addition to mechanical separation of particles by their size, **BECOPAD P** offers additional specific separation based on its cationic charge. The key factor is the positive zeta potential (electrokinetic potential), which retains negatively charged particles through adsorption.

When product flows through the cationic **BECOPAD P**, negatively charged particles accumulate at the positively charged internal surfaces. Several successive boundary layers with different charge densities are formed, the effect of which becomes weaker with increasing distance from the original charge.

The cationic **BECOPAD P** is recommended for specific reduction of negatively charged particles, for example endotoxins.

Ingredients or material of construction:
All components of the **BECOPAD P** depth filter media are FDA listed for food contact use in the Code of Federal Regulations (CFR), Title 21, 177.2260.

Components:
- Highly pure Cellulose
- Wet strength agents

2. General Characteristics of BECOPAD P Range Depth Filter Media

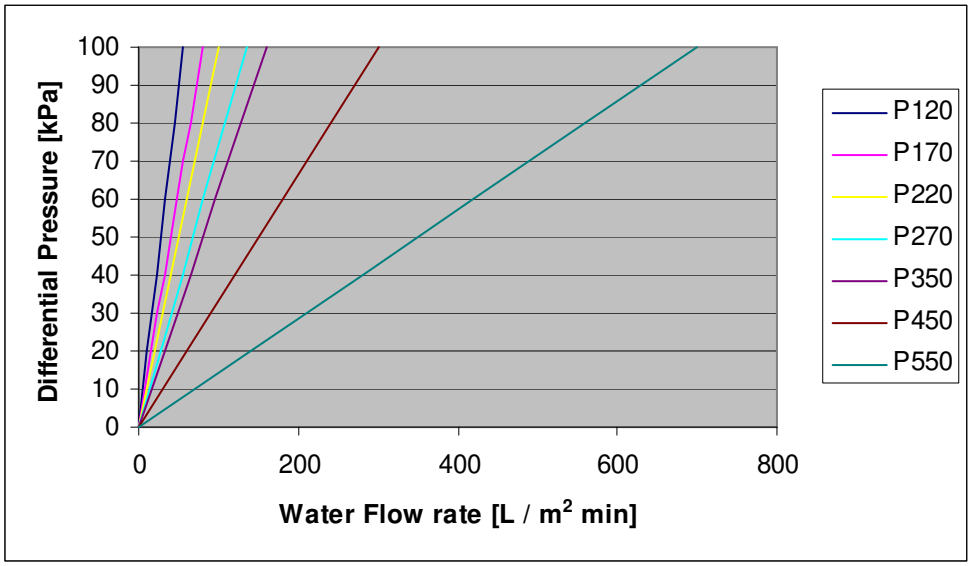
2.3 Physical data

Overview physical data

Type	Article No.	Nominal filtration rate [µm]	Thickness [mm]	Ash content [%]	Bursting strength, wet [kPa]	Water throughput at Δ p = 100 kPa [l m^{-2} min^{-1}]	Endotoxin content(1) [EU/ml]
BECOPAD P 120	Q1112	0.1 – 0.3	3.9	< 1	> 150	55	< 0.025
BECOPAD P 170	Q1117	0.2 – 0.4	3.9	< 1	> 150	80	< 0.025
BECOPAD P 220	Q1122	0.3 – 0.5	3.9	< 1	> 150	100	< 0.025
BECOPAD P 270	Q1127	0.5 – 0.7	3.9	< 1	> 150	135	< 0.025
BECOPAD P 350	Q1135	0.7 – 1.0	3.9	< 1	> 150	160	< 0.025
BECOPAD P 450	Q1145	1.0 – 2.0	3.9	< 1	> 150	300	< 0.025
BECOPAD P 550	Q1155	2.0 – 3.0	3.9	< 1	> 150	700	< 0.025

The endotoxin content is determined after rinsing with 25 l/m² of WFI.

Water Flow Rates for BECOPAD P



3. Extractable Tests

Method
In the filtration of biotech and pharmaceutical products, it is essential that product composition is not changed by filtration. An appropriate rinsing procedure after sterilization was therefore used to remove any extractable substances. The extraction media refer to the two most characteristic process conditions deionized water and additionally for the ion extractions 40 % ethanol.

For the extraction curves discussed in the following chapter, a filter with an effective filter area of 131 cm² as used. The flow rate was adjusted to 500 l/m²/h.

3. Extractable Tests

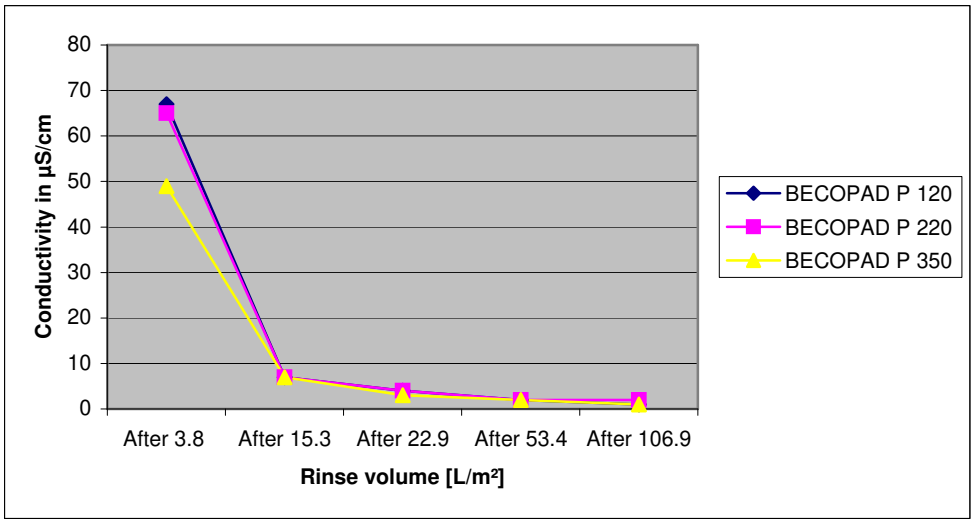
3.1 Conductivity in deionized water

Method
Conductivity is measured by a calibrated conductivity test device

Results
Conductivity Shift (in deionized water)

	Rinse volume [l/m²]				
	After 3.8	**After 15.3**	**After 22.9**	**After 53.4**	**After 106.9**
BECOPAD P 120	67	7	4	2	1
BECOPAD P 170	57	5	3	2	2
BECOPAD P 220	65	7	4	2	2
BECOPAD P 270	93	15	9	4	2
BECOPAD P 350	49	7	3	2	1
BECOPAD P 450	84	32	10	4	2
BECOPAD P 550	104	29	7	3	2

Conductivity in µS/cm at different rinsing volumes



The conductivity of the extract was < 20 µS/cm after a rinsing volume of 22.9 l/m² and can be lowered further by additional flushing.

3. Extractable Tests

3.2 Extractable Cations (Ca, Mg, Fe, Al, Cr, Cu, Zn, Cd, Mn, As, Pb) in deionized water and 40 % Ethanol

Method
For the extraction curves discussed in the following chapter, a filter with an effective filter area of 131cm² was used. The flow rate was adjusted to 500 l/m² h.

Samples were taken after a extraction volume of:

- 0 – 1.9 l/m²
- After 10 l/m²
- After 25 l/m²
- After 50 l/m²

All **BECOPAD P** depth filters are have been rinsed according to the recommendation with 25l/m² with deionized water.

All cations are measured by AAS (atomic adsorption spectroscopy) by flame or graphite tube technique.

Results
Typical ion contents for BECOPAD P

0 – 1.9 l/m²

	Deionized water*	Ethanol**
Calcium	< 1 ppm	< 1 ppm
Magnesium	< 0.5 ppm	< 0,5 ppm
Iron	< 5 ppb	< 5 ppb
Aluminum	< 5 ppb	< 5 ppb
Chromium	< 5 ppb	< 5 ppb
Copper	< 5 ppb	< 5 ppb
Zinc	< 5 ppb	< 5 ppb
Cadmium	< 5 ppb	< 5 ppb
Manganese	< 5 ppb	< 5 ppb
Arsenic	< 5 ppb	< 5 ppb
Lead	< 5 ppb	< 5 ppb

After 10 l/m²

	Deionized water*	Ethanol**
Calcium	< 25 ppb	< 25 ppb
Magnesium	< 25 ppb	< 25 ppb
Iron	< 5 ppb	< 5 ppb
Aluminum	< 5 ppb	< 5 ppb
Chromium	< 5 ppb	< 5 ppb
Copper	< 5 ppb	< 5 ppb
Zinc	< 5 ppb	< 5 ppb
Cadmium	< 5 ppb	< 5 ppb
Manganese	< 5 ppb	< 5 ppb
Arsenic	< 5 ppb	< 5 ppb
Lead	< 5 ppb	< 5 ppb

After 25 l/m²

	Deionized water*	Ethanol**
Calcium	< 25 ppb	< 25 ppb
Magnesium	< 25 ppb	< 25 ppb
Iron	< 5 ppb	< 5 ppb
Aluminum	< 5 ppb	< 5 ppb
Chromium	< 5 ppb	< 5 ppb
Copper	< 5 ppb	< 5 ppb
Zinc	< 5 ppb	< 5 ppb
Cadmium	< 5 ppb	< 5 ppb
Manganese	< 5 ppb	< 5 ppb
Arsenic	< 5 ppb	< 5 ppb
Lead	< 5 ppb	< 5 ppb

After 50 l/m²

	Deionized water*	Ethanol**
Calcium	< 25 ppb	< 25 ppb
Magnesium	< 25 ppb	< 25 ppb
Iron	< 5 ppb	< 5 ppb
Aluminum	< 5 ppb	< 5 ppb
Chromium	< 5 ppb	< 5 ppb
Copper	< 5 ppb	< 5 ppb
Zinc	< 5 ppb	< 5 ppb
Cadmium	< 5 ppb	< 5 ppb
Manganese	< 5 ppb	< 5 ppb
Arsenic	< 5 ppb	< 5 ppb
Lead	< 5 ppb	< 5 ppb

Conclusion
All evaluated ions are extracted in on a very low level. This apply for deionized water as well as for 40 % ethanol.

3. Extractable Tests

3.3 Total Extractables according to the guideline of FDA CFR 21 Chapter 177.2260

Method
Under Title 21 «Food and Drugs» (2), Chapter 177 the Code of Federal Regulations of the FDA (Food and Drug Administration) is concerned with indirect food additives: polymers. Subpart C. Substances for use only as components of articles intended for repeated use.
The 177.2260 refers to resin-bonded filters and states the limits for extractables in different extraction media and under different extraction conditions. The following limitations are stated here.

Extraction Solvent	Extraction Conditions	CFR Limits
deionized Water	2 h/boiling heat	< 4 % dry residue
50 % ethanol	2 h/60 °C	< 4 % dry residue
8 % ethanol	2 h/80 °C	< 4 % dry residue
5 % acetic acid	2 h/90 °C	< 4 % dry residue
n-hexane	2 h/boiling heat	< 0.5 % dry residue

Although these regulations are not primarily intended for pharmaceutical products, they provide additional supporting data for the suitability of filters for pharmaceutical applications.

Results and Conclusion
The level of extractables of all BEGEROW depth filter media was significantly below the CFR limits and therefore meets the requirements of 21 CFR 177.2260.
For a detailed report please refert to Appendix I.

3.4 Investigation according to LFGB and recommendation XXXVI/1 of the BfR

Method
BECOPAD P meet the requirements of LFGB (German Food, Commodity, and Feed Act), and the Recommendation XXXVI/1 issued by BfR (German Federal Institute of Risk Assessment).
This test report includes:
Hot water extracts for the determination of:

Formaldehyde	< 0.3 mg/g
Heavy metals:	
Lead	< 0.3 µg/g
Cadmium	< 0.5 µg/g
Mercury	< 0.3 µg/g
Chromium	not detectable
Total Dry Residue	< 5 mg/g
Total Nitrogen	< 3 mg/g dry substance

Sensory tests
Germ Growth Inhibition Tests
Detection of the migration of Dichloropropanol < 2 µg/g
Monochloropropandiol < 12 µg/g

Results and Conclusion
BECOPAD P depth filters pass all tests according the requirements LFGB.
A detailed report and further validation information are available by contacting BEGEROW.

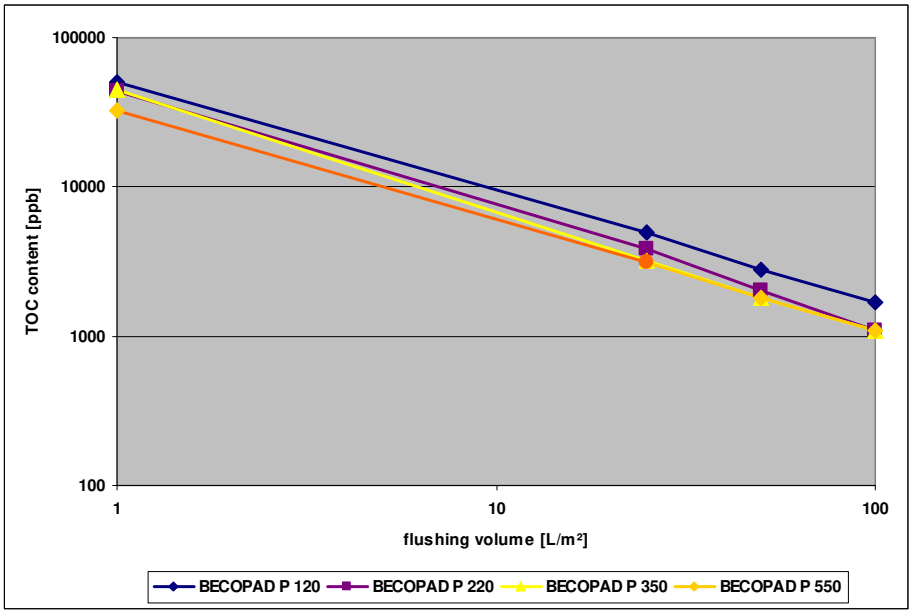
3. Extractables Test

3.5 Total Organic Carbon

Method
To determine the quantity of organic extractables in BECOPAD the depth filter media were tested for total organic carbon (TOC).

Four BECOPAD P depth filter types have been tested exemplary for the whole range The filters with an area of 131 cm^2 m^2 have been flushed with deionized water at a flow rate of 500 L / m^2 h. Samples of flushing water were taken after 1 L, 25 L, 50 L and 100 L and analyzed for TOC contents.

Result
TOC values in flushing water in ppb



3. Extractables Test

3.6 Oxidizable Substances

All BECOPAD P depth filter types have been investigated for the presence of oxidizable substances in the filtrate as described in the current USP.

Method
Samples of BECOPAD P depth filter media have been inserted into a stainless steel filter holder and flushed with destilled water at a flow rate of 500 l/m^2 min. Samples with a volume of 100 ml were taken in 25 l/m^2 steps up to a total flushing volume of 200 l/m^2.

The detection of the presence of oxidizable substances has been conducted acc. to the current USP.

Result
Oxidizable substances in filtrate of BECOPAD P depth filter media.

Flushing volume [L/m^2]	BECOPAD P 120	BECOPAD P 170	BECOPAD P 220	BECOPAD P 270	BECOPAD P 350	BECOPAD P 450	BECOPAD P 550
25	passed	passed	passed	passed	passed	passed	passed
50	passed	passed	passed	passed	passed	passed	passed
75	passed	passed	passed	passed	passed	passed	passed
100	passed	passed	passed	passed	passed	passed	passed
125	passed	passed	passed	passed	passed	passed	passed
150	passed	passed	passed	passed	passed	passed	passed
175	passed	passed	passed	passed	passed	passed	passed
200	passed	passed	passed	passed	passed	passed	passed

4. Endotoxins

In a medical sense, pyrogen means fever pathogen. Examples are Endotoxins of gram-negative bacteria. These are cell wall constituents that consist of a lipid and a polysaccharide component and are embedded in the outer membrane of gran-negative microbes. Quantitative proof of Endotoxins can be established using the LAL test (**L**imulus **A**moebocyte **L**ysate) which is also used for the routine examination of the **BECOPAD P** depth filters.

Methods
The Endotoxin content of the samples is specified in EU/ml (Endotoxin unit) testing with the kinetic turbidimetric LAL method. The sample is incubated with the LAL reagent and the rate of the increase in turbidity during the time is taken. It is measured spectrophotometrically and compared to a standard curve. Prior to the measurement of the Endotoxin content, depth filters are rinsed with 25 l/m² WFI. The tests are performed by a accredited laboratory according to the guidelines of Ph.Eur. 2.6.14 [harmonized testing].

Results

Type	Article No.	Endotoxin content(1) [EU/ml]
BECOPAD P 120	Q1112	< 0.025
BECOPAD P 170	Q1117	< 0.025
BECOPAD P 220	Q1122	< 0.025
BECOPAD P 270	Q1127	< 0.025
BECOPAD P 350	Q1135	< 0.025
BECOPAD P 450	Q1145	< 0.025
BECOPAD P 550	Q1155	< 0.025

(1) The endotoxin content is determined after rinsing with 25 l/m² of WFI.

Conclusion
Endotoxin limit values at < 0.025 EU/ml are defined for the **BECOPAD P** range.

5. Bacterial Challenge Testing

Fine depth filters have the potential to reduce the bioburden load in a solution. To evaluate their bioburden reduction the depth filter is challenged with a defined bacteria suspension and the titer reduction in the filtrate is determined.

The reduction capability of depth filters is influenced by several factors such as the physical and chemical properties of the fluid, the process parameters and the type of challenge organism.

Result
Log. Reduction values for BECOPAD P depth filter media.

Type	Nominal retention rate [µm]	Log Reduction Value [LRV]
BECOPAD P 120	**0.1 – 0.3**	**8.0**[1]
BECOPAD P 170	**0.2 – 0.4**	**6.7**[1]
BECOPAD P 220	**0.3 – 0.5**	**5.9**[2]
BECOPAD P 270	**0.5 – 0.7**	**5.1**[2]
BECOPAD P 350	**0.7 – 1.0**	**3.5**[2]

[1] Challenged with *Brevundimonas diminuta* (ATCC 19146)
[2] Challenged with *Serratia marcescens* (ATCC 14765)

6. Chemical Data

The **BECOPAD P** depth filters are tested by putting them under the written temperature for at maximum 168 h in the chemical compound. If the time is below 168 h, this means that the depth filter was monitored as resistant at the maximum given value of time.

Chemical resistance of BECOPAD P depth filter media

Chemical compound		Max. tested temperature/ Contact time	Mechanical resistance
Caustic:			
ammonia solution	25%	20 °C/168 h	X
potassium hydroxide	30%	20 °C/ 48 h	(X)
sodium hydroxide	30%	20 °C/ 24 h	-
	5%	40 °C/ 4 h	X
	2%	40 °C/ 4 h	X
	1%	40 °C/ 4 h	X
	0.5%	40 °C/ 4 h	X
Acids:			
acetic acid	25%	20 °C/168 h	X
peracetic acid	0.1%	20 °C/168 h	X
	0.2%	20 °C/ 168 h	X
	0.5%	20 °C/ 168 h	X
nitric acid	25%	20 °C/ 48 h	X
hydrochloric acid	25%	20 °C/168 h	X
sulphuric acid	25%	20 °C/ 48 h	X
citric acid	25%	20 °C/168 h	X

Chemical compound		Max. tested temperature/ Contact time	Mechanical resistance
Organic solvents:			
acetone		20 °C/168 h	X
butanol		20 °C/168 h	X
cyclohexane		20 °C/168 h	X
dimethyl sulphide		20 °C/168 h	X
ethanol		20 °C/168 h	X
ethylene glycol		20 °C/168 h	X
ethyl methyl ketone		20 °C/168 h	X
isopropanol		20 °C/168 h	X
methanol		20 °C/168 h	X
N,N dimethyl formamide		20 °C/168 h	X
n-hexane		20 °C/168 h	X
tetrachloroethylene		20 °C/168 h	X
toluene		20 °C/168 h	X
triethanolamine		20 °C/168 h	X
xylene		20 °C/168 h	X
Aqueous solutions:			
iron trichloride	25%	20 °C/168 h	X
sodium hypochlorite free chlorine	12%	20 °C/168 h	X
hydrogen peroxide	10%	20 °C/ 72 h	X

X = resistant **(X) = limited resistance** **- = not resistant**

7. Biological Safety

BECOPAD P depth filter media have been evaluated for their biological properties. According to the following guidelines the depth filter media have been examined for cytotoxicity, systemic cytotoxicity, irritation and tissue compatibility:

- Cytotoxicity in accordance to DIN EN ISO 10993-5:1999
- USP Plastic Class VI test in accordance to USP 32 <88>

Method
In the Cytotoxicity Test extracts of **BECOPAD P** depth filter media are investigated for any growth inhibition of L929 mouse fibroblasts. All extracts of **BECOPAD P** depth filter media did not show any growth inhibition effect.

Further the **BECOPAD P** depth filter media have been tested according to USP Plastic Class VI test in systemic injection test, intracutaneous reactivity test and implantation test for their biological safety in test animals. In all test animals no effects were observed.

Result
Based on the test results described above it can be stated that **BECOPAD P** depth filter media have no cytotoxic potential and comply with the requirements of USP Plastic Class VI test in accordance to USP 32 <88>.

For details see Appendix II.

8. Appendix

8.1 Appendix I (14 pages)

Test reports “Total extractables acc. to the guideline of FDA CFR 21 Chapter 177.2260”
Test reports acc. to LFBG

8.2 Appendix II (7 pages)

Test report “Cytotoxicity in accordance to DIN EN ISO 10993-5:1999”
Test report “USP Plastic Class test in accordance to USP 32 <88>”