

Biopur® – A Unique Dimension in Biological Purity

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Introduction

The certified Biopur® standard has been created to ensure the highest possible purity of consumables for most demanding applications and to meet the highest demands, where protection against different kind of contaminations

is crucial. In this report, we present background information about parameters that are of critical importance for the purity of these consumables.

Plastic consumables used in the laboratory which come into direct contact with sample material must not compromise the sample nor influence the results of analyses performed with these consumables. High purity of consumables is particularly important for applications which include living cells or nucleic acids. Many consumables can be sterilized by autoclaving, but molecules such as DNA, RNases and endotoxins are very stable and cannot be removed entirely, or inactivated, by this method. Hence, the availability of ready-to-use sterile products which are also free of molecules relevant in molecular or cell biology is advantageous. These products can be used directly, thus saving time and money while ensuring highest safety.

Twenty years ago, Eppendorf created a unique purity grade for consumables, Biopur, the testing criteria of which were expanded in 2012 to include the absence of DNase and PCR inhibitors. Pipette tips, Safe-Lock Tubes, Conical Tubes and Combitips bearing the Biopur seal are produced in a process with extensive automation of all production steps. Contamination with nucleic acids, proteins or microorganisms by human contact or through other sources is thereby excluded. In addition, sterilization of products is ensured by irradiation.

Every batch is tested by an independent laboratory; only batches fulfilling all test criteria will be certified. The certificate (Fig. 1) serves to guarantee the purity of all Biopur products from Eppendorf, listing the threshold limits for sterility, pyrogens (endotoxins), DNA, DNase, RNase, ATP and PCR-inhibitors.

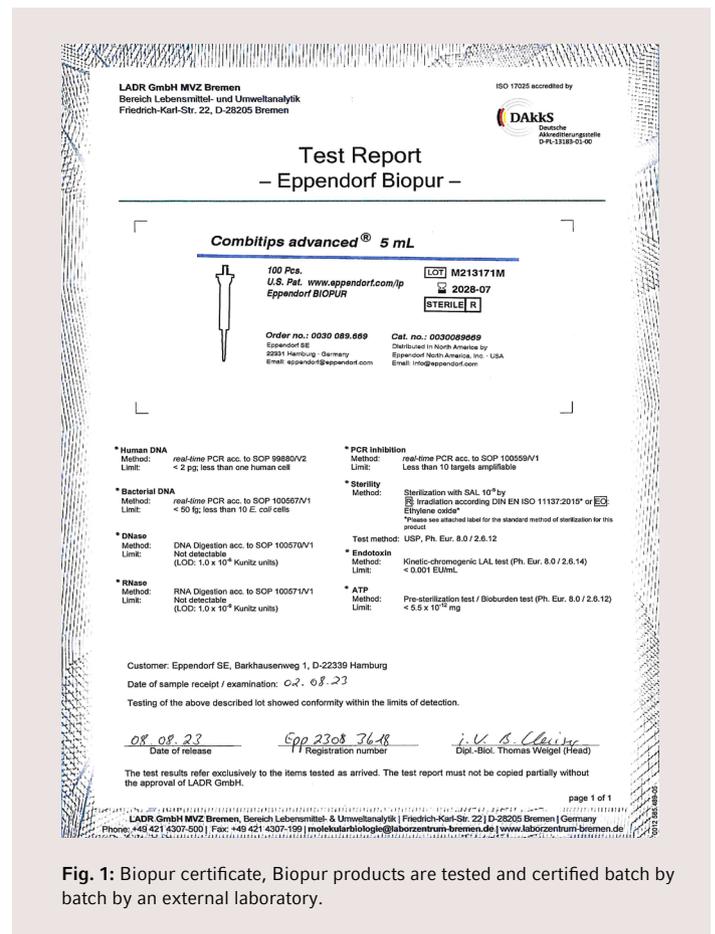


Fig. 1: Biopur certificate, Biopur products are tested and certified batch by batch by an external laboratory.

The batch specific certificates are available at www.eppendorf.com/certificate

Biopur seal

The Biopur seal (Fig. 2) stands for the reliability with which the product can be used directly for all applications requiring highest purity. All products with this seal are guaranteed:

- > Sterile (high energy electron radiation SAL of 10^{-6})
- > Pyrogen-free (< 0.001 EU/ml, kinetic turbidimetric LAL-test, FDA guideline)
- > ATP-free (< 5.5 fg)
- > RNase-free (< 1.0×10^{-9} Kunitz units)
- > DNase-free (< 1.0×10^{-6} Kunitz units)
- > Human DNA-free (< 2 pg; less than one human cell)
- > Bacterial DNA-free (< 50 fg; less than 10 *E. coli* cells)
- > PCR-inhibitor-free (less than 10 amplifiable targets)

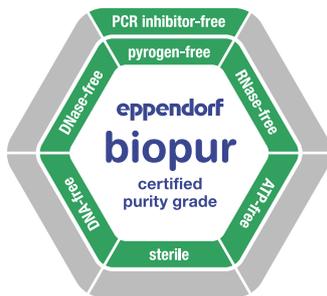


Fig. 2: Biopur seal

Sterility

In accordance with an initiative of the European Confederation of Medical Suppliers Associations (EUCOMED), a contamination probability with viable microorganisms of 10^{-6} after a sterilization process was specified for reliable sterilization. This is equivalent to a Sterility Assurance Level (SAL) of 10^{-6} , which means: 1 microorganism surviving in a sample of 10^6 or 1 non-sterile item after sterilization of a batch of 10^6 items.

For Biopur products, sterility with an SAL of 10^{-6} is achieved by irradiation. The choice of radiation dose is based on experimental determination of bioburden and on sterility tests. This process reflects the high quality of the entire production process. Following validation of the sterilization process and sterility of the product by using suitable biological

indicators (*Bacillus pumilus*), the subsequent process is monitored with dosimeters, in accordance with USP XXII (United States Pharmacopoeia), DAB 10 (German Pharmacopoeia) and Ph. Eur. (European Pharmacopoeia).

Sterilization via radiation can be achieved with either beta irradiation (accelerated electrons) or gamma irradiation (high energy electromagnetic rays). Of note, both types of irradiation, depending on the dose, may influence material composition, and thus the mechanical properties of plastic products. Biopur products are produced under fully automated cleanroom conditions, without human intervention. The low bioburden hereby achieved allows for the radiation dose required for sterilization to be set at a very low level.

For sterility testing of samples, the Eppendorf products are rinsed in thioglycolate and CaSo-bouillon and incubated for 15 days. The test is performed in accordance with Ph. Eur. 2.6.1. "Sterility testing".

Pyrogens

Pyrogens (from greek: pyr, pyros = fire; genes = to arise) Pyrogens form a class of substances that cause fever when given parenterally. Endotoxins as the most important class of pyrogens are monitored in Biopur products by a kinetic-turbidimetric LAL-Test. LAL is the abbreviation for Limulus Amoebocyte Lysate. This lysate of blood cells from *Limulus polyphemus* ("horseshoe crab", Figure 3) reacts very sensitively to very small amounts of the lipopolysaccharide fraction of bacterial cell envelopes of gram-negative bacteria (bacterial endotoxins). The addition of a solution containing endotoxins to a solution of the lysate produces turbidity, precipitation or gelation of the mixture.

Horseshoe crabs are used as blood donors for the collection of amoebocytes. Fortunately, the crabs do not have to be killed for this procedure. The Ph. Eur. (European Pharmacopoeia) and also the FDA developed guidelines for the use of gel tests and kinetic-turbidimetric tests with LAL in 1987 and 1988, respectively.

The LAL-Test for Biopur products is carried out with endotoxin standards (i.e. WHO-Standard 84/650 or Endotoxin BRS) or control standard endotoxins. To this end, the products are rinsed with endotoxin-free water, and the test is performed in accordance with the Ph. Eur. 2.6.14 "Testing for bacterial endotoxins".

ATP

ATP can be used as a general indicator for the presence of a biological contamination as it is an energy-rich molecule that is part of all living cells. Biopur products are free of ATP which is tested via a bioburden test and therefore, they are optimally suited for the use with ATP quantification kits. These kits commonly use the enzyme luciferase from firefly organs, which catalyzes the formation and oxidation of adenylyl-luciferin with the emission of light.

This bioluminescence is used as an indicator for metabolic activities or the presence of biological materials.



Fig. 3: North American horseshoe crab *Limulus polyphemus*

DNA, DNases and RNases

Contaminating nucleic acids and enzymes that cleave DNA or RNA are critical substances which can have considerable influence on experimental work in molecular biology, e.g. Polymerase Chain Reaction (PCR) or other amplification techniques. Contaminations of plastic consumables during production with DNA, DNases and RNases are mostly due to human contact and air-borne pollutants. These occurrences are precluded from the entire Biopur production process.

For testing of the Biopur products, these were rinsed with nucleic acid-free and nuclease-free water which was subsequently subjected to the following analyses: Determination of DNA is performed via real-time PCR. Contamination with bacterial DNA is indicated by amplification of a highly conserved 110 bp fragment from the 16S rDNA. For human DNA, a 294 bp fragment, present in more than 10^5 copies per cell, is amplified. A serial dilution of 16 pg – 2 pg of this DNA fragment serves as positive control and comparison standard (Fig. 4).

The presence of DNases or RNases is examined by incubation of the rinsing solution with a 100 bp DNA ladder or a 100 b RNA ladder, respectively, at 37 °C for 24 h and subsequent analysis via gel electrophoresis.

PCR inhibitors

There are substances which disrupt the amplification of DNA during PCR. In order to detect possible PCR inhibitors in Biopur products, these are rinsed with DNA-free water. This rinse is then used in a real-time PCR setup to amplify a 294 bp fragment which is present in 105 copies in one human cell. The C_t -values are compared to the positive control (containing 16 pg human DNA). In order to pass, the difference in C_t -values must not exceed ± 2 cycles.

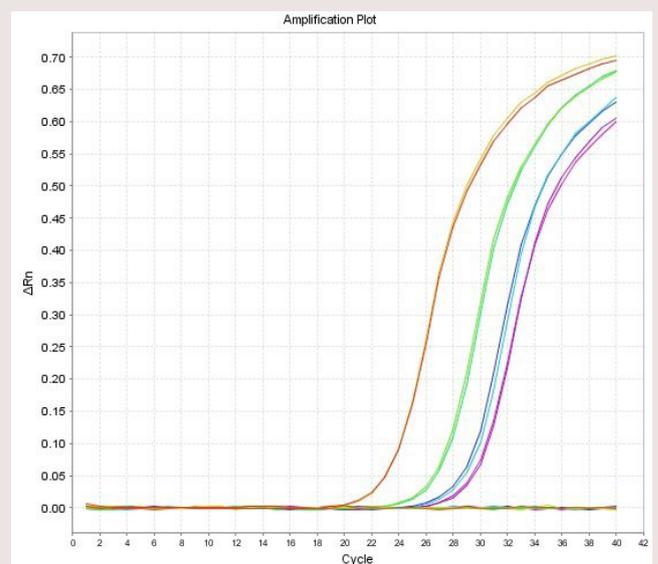


Fig. 4: Real-time PCR of a serial dilution of human DNA.

The figure shows 4 fluorescence double curves which correspond to the double preparations of human DNA serial dilutions (from left to right = 16, 8, 4, 2 pg L1-DNA).

Ordering information

Description	Order no.
epT.I.P.S.[®] Singles, Biopur[®] , individually wrapped	
0.1–20 µL, 40 mm, 100 pcs.	0030 010 027
2–200 µL, 53 mm, 100 pcs.	0030 010 043
50–1,000 µL, 71 mm, 100 pcs.	0030 010 060
epT.I.P.S.[®] Racks, Biopur[®]	
0.1–20 µL, 40 mm, 5 racks of 96 tips	0030 075 226
2–200 µL, 53 mm, 5 racks of 96 tips	0030 075 234
20–300 µL, 55 mm, 5 racks of 96 tips	0030 075 242
50–1,000 µL, 71 mm, 5 racks of 96 tips	0030 075 250
50–1,250 µL, 76 mm, 5 racks of 96 tips	0030 075 269
50–1,250 µL L, 103 mm, 5 racks of 96 tips	0030 075 277
250–2,500 µL, 115 mm, 5 racks of 48 tips	0030 075 285
0.1–5 mL, 120 mm, 5 racks of 24 tips	0030 075 293
0.2–5 mL, 175 mm, 5 racks of 24 tips	0030 075 307
0.5–10 mL, 165 mm, 5 racks of 24 tips	0030 075 315
epT.I.P.S.[®] BioBased Reloads Biopur[®]	
0.1–20 µL, 40 mm, 5 reloads of 96 tips	0030 075 420
2–200 µL, 53 mm, 5 reloads of 96 tips	0030 075 439
20–300 µL, 55 mm, 5 reloads of 96 tips	0030 075 447
50–1,000 µL, 71 mm, 5 reloads of 96 tips	0030 075 455
50–1,250 µL, 76 mm, 5 reloads of 96 tips	0030 075 463
50–1,250 µL L, 103 mm, 5 reloads of 96 tips	0030 075 471
epDualfilter T.I.P.S.[®] SealMax[®] BioBased Reloads Biopur[®]	
0.5–20 µL L, 45,7 mm, 10 reloads of 96 tips	0030 081 234
2–100 µL, 52,7 mm, 10 reloads of 96 tips	0030 081 242
2–200 µL, 55,2 mm, 10 reloads of 96 tips	0030 081 250
20–300 µL, 55 mm, 10 reloads of 96 tips	0030 081 269
50–1,000 µL, 75,5 mm, 10 reloads of 96 tips	0030 081 277
Combitips advanced[®], Biopur[®], 100 pcs. , individually blister-wrapped	
0.1 mL, white, 100 pcs.	0030 089 618
0.2 mL, light blue, 100 pcs.	0030 089 626
0.5 mL, purple, 100 pcs.	0030 089 634
1 mL, yellow, 100 pcs.	0030 089 642
2.5 mL, green, 100 pcs.	0030 089 650
5 mL, blue, 100 pcs.	0030 089 669
10 mL, orange, 100 pcs.	0030 089 677
25 mL, red, 100 pcs.	0030 089 685
50 mL, light grey, 100 pcs.	0030 089 693
25 mL adapter, red, 7 pcs.	0030 089 731
50 mL adapter, light grey, 7 pcs.	0030 089 740
Eppendorf Safe-Lock Tubes, Biopur[®]	
0.5 mL, 50 pcs.	0030 121 570
1.5 mL, 100 pcs.	0030 121 589
2.0 mL, 100 pcs.	0030 121 597
Eppendorf Tubes[®] 5.0 mL, Biopur[®] , individually wrapped	
5.0 mL, 50 pcs.	0030 119 479

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