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Eppendorf Biopur®–A Unique Dimension in Biological Purity

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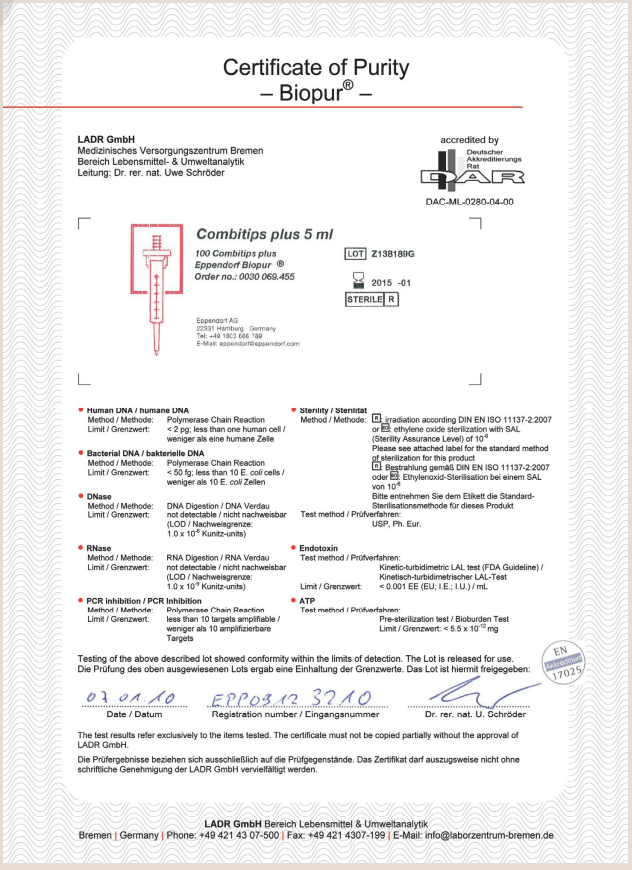
The certified Eppendorf Biopur standard has been created to ensure the highest possible purity of consumables for most demanding applications and to meet the highest demands, where protection against different kind of contaminations is crucial. In this report, we present background information about 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, Eppendorf Biopur, the testing criteria of which were expanded in 2012 to include the absence of DNase and PCR inhibitors. Pipette tips, Safe-Lock Tubes, twin.tec PCR plates and Combitips bearing the Eppendorf 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 either irradiation or ethylene oxide treatment.

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 Eppendorf Biopur products from Eppendorf, listing the threshold limits for sterility, pyrogens (endotoxins), DNA, DNase, RNase, ATP and PCR-inhibitors.



Certificate of Purity
– Biopur® –

LADR GmbH
Medizinisches Versorgungszentrum Bremen
Bereich Lebensmittel- & Umweltanalytik
Leitung: Dr. rer. nat. Uwe Schröder

accredited by Deutscher Akkreditierungs Rat
DAC-ML-0280-04-00

Combitips plus 5 ml
100 Combitips plus
Eppendorf Biopur ®
Order no.: 0030 069.455

LOT Z138189G
2015 -01
STERILE R

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- Human DNA / humane DNA
  Method / Methode: Polymerase Chain Reaction
  Limit / Grenzwert: < 2 pg; less than one human cell / weniger als eine humane Zelle
- Bacterial DNA / bakterielle DNA
  Method / Methode: Polymerase Chain Reaction
  Limit / Grenzwert: < 50 fg; less than 10 E. coli cells / weniger als 10 E. coli Zellen
- DNase
  Method / Methode: DNA Digestion / DNA Verdau
  Limit / Grenzwert: not detectable / nicht nachweisbar (LOD / Nachweisgrenze: 1.0×10^{-6} Kunitz-units)
- RNase
  Method / Methode: RNA Digestion / RNA Verdau
  Limit / Grenzwert: not detectable / nicht nachweisbar (LOD / Nachweisgrenze: 1.0×10^{-6} Kunitz-units)
- PCR inhibition / PCR Inhibition
  Method / Methode: Polymerase Chain Reaction
  Limit / Grenzwert: less than 10 targets amplifiable / weniger als 10 amplifizierbare Targets
- Sterility / Sterilität
  Method / Methode: X irradiation according DIN EN ISO 11137-2:2007 or ☐ ethylene oxide sterilization with SAL (Sterility Assurance Level) of 10^{-6}
  Please see attached label for the standard method of sterilization for this product
  X Bestrahlung gemäß DIN EN ISO 11137-2:2007 oder ☐ Ethylenoxid-Sterilisation bei einem SAL von 10^{-6}
  Bitte entnehmen Sie dem Etikett die Standard-Sterilisationsmethode für dieses Produkt
  Test method / Prüfverfahren: USP, Ph. Eur.
- Endotoxin
  Test method / Prüfverfahren: Kinetic-turbidimetric LAL test (FDA Guideline) / Kinetisch-turbidimetrischer LAL-Test
  Limit / Grenzwert: < 0.001 EE (EU; I.E.; I.U.) / mL
- ATP
  Test method / Prüfverfahren: Pre-sterilization test / Bioburden Test
  Limit / Grenzwert: < 5.5 x 10^{-12} mg

Testing of the above described lot showed conformity within the limits of detection. The Lot is released for use.
Die Prüfung des oben ausgewiesenen Lots ergab eine Einhaltung der Grenzwerte. Das Lot ist hiermit freigegeben:

07.01.10 — Date / Datum
EPP08.12 3210 — Registration number / Eingangsnummer
Dr. rer. nat. U. Schröder — Dr. rer. nat. U. Schröder

EN Accredited 17025

The test results refer exclusively to the items tested. The certificate must not be copied partially without the approval of LADR GmbH.
Die Prüfergebnisse beziehen sich ausschließlich auf die Prüfgegenstände. Das Zertifikat darf auszugsweise nicht ohne schriftliche Genehmigung der LADR GmbH vervielfältigt werden.

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

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

Eppendorf Biopur seal

The Eppendorf 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 or treatment with ethylene oxide, SAL of 10^{-6})
> Pyrogen-free (< 0.001 EU/ml, kineticturbidimetric LAL-test, FDA guideline)
> ATP-free (< 5.5 fg)
> RNase-free (< 1.0 x 10^{-9} Kunitz units)
> DNase-free (< 1.0 x 10^{-6} Kunitz units) – new!
> 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)

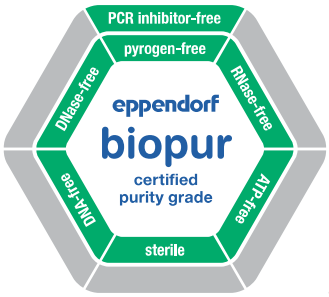

Fig. 2: Eppendorf Biopur seal

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 Eppendorf Biopur products, sterility with an SAL of 10^{-6} is achieved by irradiation or treatment with ethylene oxide (EtO). The choice of sterilization 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 betair-radiation (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. Eppendorf 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.

An additional very effective sterilization method is treatment with ethylene oxide, which is frequently used for medicinal products in hospitals. The advantage of this method is the fact that the mechanical properties of the plastic are not affected. Strict adherence to the process and a sufficiently long ventilation period of the sterilized products are crucial. 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 Eppendorf 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 Pharmacepoeia) 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 Eppendorf 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".

Fig. 3: North American horseshoe crab Limulus polyphemus

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. Eppendorf 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 adenyl-luciferin with the emission of light. This bioluminescence is used as an indicator for metabolic activities or the presence of biological materials.

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 Eppendorf Biopur production process.

For testing of the Eppendorf 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 Eppendorf 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 Ct-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.

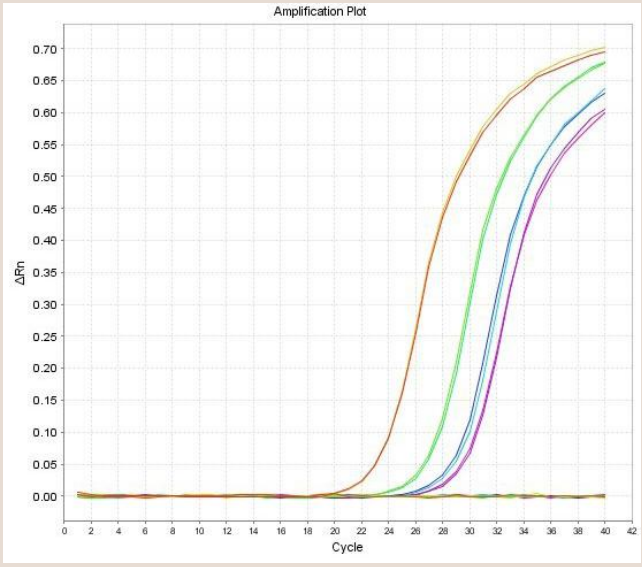

Fig. 4: Real-time PCR of a serial dilution of human DNA.
The figure shows 4 fluorescence double curves which correspond to the double preparations of human DNA serial dilutions (from left to right = 16, 8, 4, 2 pg L1-DNA).

Ordering information

Description	Order no. international	Order no. North America
epT.I.P.S.® Singles, Eppendorf Biopur®, individually wrapped		
0.1–20 µL, 40 mm, 100 pcs.	0030 010.019	022491130
2–200 µL, 53 mm, 100 pcs.	0030 010.035	022491148
50–1,000 µL, 71 mm, 100 pcs.	0030 010.051	022491156
epT.I.P.S.® Racks, Eppendorf Biopur®		
0.1–20 µL, 40 mm, 5 racks of 96 tips	0030 075.005	022491067
2–200 µL, 53 mm, 5 racks of 96 tips	0030 075.021	022491083
20–300 µL, 55 mm, 5 racks of 96 tips	0030 075.048	022491091
50–1,000 µL, 71 mm, 5 racks of 96 tips	0030 075.064	022491105
50–1,250 µL, 76 mm, 5 racks of 96 tips	0030 075.080	022491113
50–1,250 µL L, 103 mm, 5 racks of 96 tips	0030 075.129	022494014
500–2,500 µL, 115 mm, 5 racks of 48 tips	0030 075.102	022491121
1–10 mL, 165 mm, 5 racks of 24 tips	0030 075.145	022491164
Eppendorf Combitips advanced®, Eppendorf Biopur®, individually wrapped		
0.1 mL, white, 100 pcs.	0030 089.618	0030089618
0.2 mL, light blue, 100 pcs.	0030 089.626	0030089626
0.5 mL, violet, 100 pcs.	0030 089.634	0030089634
1 mL, yellow, 100 pcs.	0030 089.642	0030089642
2.5 mL, green, 100 pcs.	0030 089.650	0030089650
5 mL, blue,100 pcs.	0030 089.669	0030089669
10 mL, orange, 100 pcs.	0030 089.677	0030089677
25 mL, red, 100 pcs.	0030 089.685	0030089685
50 mL, light grey, 100 pcs.	0030 089.693	0030089693
25 mL adapter, red, 7 pcs.	0030 089.731	0030089731
50 mL adapter, light grey, 7 pcs.	0030 089.740	0030089740
Eppendorf Safe-Lock Tubes, Eppendorf Biopur®		
0.5 mL, 50 pcs.	0030 121.570	022600001
1.5 mL, 100 pcs.	0030 121.589	022600028
2.0 mL, 100 pcs.	0030 121.597	022600044
Eppendorf Tubes® 5.0 mL, Eppendorf Biopur®, individually wrapped		
5.0 mL, 50 pcs.	0030 119.479	0030119479
Eppendorf twin.tec® microbiology PCR Plate 96, skirted, Eppendorf Biopur®, Set of 10		
clear	0030 129.300	0030129300
blue	0030 129.318	0030129318
Eppendorf twin.tec® microbiology PCR Plate 96, semi-skirted, Eppendorf Biopur®, Set of 10		
clear	0030 129.326	0030129326
blue	0030 129.334	0030129334
Eppendorf twin.tec® microbiology PCR Plate 384, Eppendorf Biopur®, Set of 10		
clear	0030 129.342	0030129342
blue	0030 129.350	0030129350

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