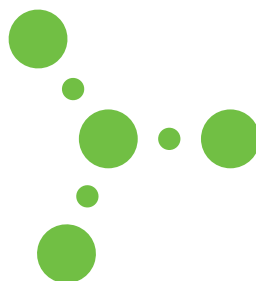


dNTP Guide

Deoxynucleotides



- ▶ *Primary manufacturer with facilities in Germany*
- ▶ *ISO 9001:2000 certified Quality Management System*
- ▶ *From micro-litres to multi-litres to lyophilisates*
- ▶ *Free lot-specific qualification samples*
- ▶ *Single lot reservations for up to 12 months*
- ▶ *Individual delivery schedules*
- ▶ *Custom testing and documentation*
- ▶ *Custom formulations, packaging & labeling*



Jena Bioscience
www.jenabioscience.com

Jena Bioscience

Company profile



IFTA AG
Certified Quality Management System
according to DIN EN ISO 9001:2000-12
Registry No.: IC 03214 034

Jena Bioscience GmbH was founded by a team of scientists from the Max-Planck-Institute for Molecular Physiology in Dortmund. 25+ years of academic know how were condensed into the company in order to develop innovative reagents and technologies for the life science market.

Since the start up in 1998, the company has evolved into an established global reagent supplier with more than 3000 products on stock and a customer base in 50+ countries. Jena Bioscience serves three major client groups:

- **Research laboratories at universities, industry, government, hospitals and medical schools**
- **Pharmaceutical industry in the process from lead discovery through to pre-clinical stages**
- **Laboratory & diagnostic reagent kit producers and re-sellers**

Our company premises are located in the city of Jena / Germany with a subsidiary in Teltow, in the vicinity of the German capital Berlin.



Jena Bioscience's products include nucleosides, nucleotides and their non-natural analogs, recombinant proteins & protein production systems, reagents for the crystallization of biological macromolecules and tailor-made solutions for molecular biology and biochemistry.

In our chemistry division, we have hundreds of natural and modified nucleotides available on stock. In addition, with our pre-made building blocks and in-house expertise we manufacture even the most exotic nucleotide analog from the mg to kg scale.

In the field of recombinant protein production, Jena Bioscience has developed its proprietary LEXSY technology. LEXSY (Leishmania Expression System) is based on a S1-classified unicellular organism that combines easy handling with a full eukaryotic protein folding and modification machinery including mammalian-like glycosylation. LEXSY is primarily used for the expression of proteins that are expressed at low yields or are inactive in the established systems, and expression levels of up to 300 mg/L of culture were achieved.

For the crystallization of biological macromolecules – which is the bottleneck in determining the 3D-structure of any protein – we offer specialized reagents for crystal screening, crystal optimization and phasing that can reduce the time for obtaining high quality crystals suitable for X-ray diffraction from several years to a few days.

Our specialized reagents are complemented with a large selection of products for any molecular biology & biochemistry laboratory such as kits for Standard PCR and Real-Time PCR, oligonucleotides, cloning enzymes, mutagenesis technologies, and many more...

We combine highest quality standards for all our products with individualized customer support. We establish direct lines of communication from clients to our in-house scientists, resulting in productive interactions among people with similar and research interests who speak the same language. Furthermore, we offer support programs and attractive discount schemes for young scientists establishing their own labs. If you wish to receive more information on Jena Bioscience, just send us an E-mail to info@jenabioscience.com.



dNTP Guide



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Deoxynucleotides (dNTPs)

Specifications

	dATP; 100 mM Sodium Salt Solution	dCTP; 100 mM Sodium Salt Solution	dGTP; 100 mM Sodium Salt Solution	dTTP; 100 mM Sodium Salt Solution	dUTP; 100 mM Sodium Salt Solution
Nomenclature	2'-Deoxyadenosine 5'-triphosphate	2'-Deoxycytidine 5'-triphosphate	2'-Deoxyguanosine 5'-triphosphate	2'-Deoxythymidine 5'-triphosphate	2'-Deoxyuridine 5'-triphosphate
CAS Number	1927-31-7	102783-51-7	93919-41-6	18423-43-3	102814-08-4
Catalog Number	NU-1001	NU-1002	NU-1003	NU-1004	NU-1008
Formula (Anion)	$C_{10}H_{13}N_5O_{12}P_3$	$C_9H_{13}N_3O_{13}P_3$	$C_{10}H_{13}N_5O_{13}P_3$	$C_{10}H_{14}N_2O_{14}P_3$	$C_9H_{12}N_2O_{14}P_3$
Formula Weight (g × mol⁻¹)	488.16	464.13	504.16	479.14	465.12
Storage	at -20 °C	at -20 °C	at -20 °C	at -20 °C	at -20 °C
Stability	24 months from certification date	24 months from certification date	24 months from certification date	24 months from certification date	24 months from certification date
Appearance	clear colorless solution	clear colorless solution	clear colorless solution	clear colorless solution	clear colorless solution
Concentration ⁽¹⁾ (22 °C, pH 7.0)	100-110 mM ($\epsilon = 15.1 \text{ l} \times \text{mmol}^{-1} \times \text{cm}^{-1}$, 259 nm)	100-110 mM ($\epsilon = 8.9 \text{ l} \times \text{mmol}^{-1} \times \text{cm}^{-1}$, 271 nm)	100-110 mM ($\epsilon = 14.2 \text{ l} \times \text{mmol}^{-1} \times \text{cm}^{-1}$, 252 nm)	100-110 mM ($\epsilon = 9.5 \text{ l} \times \text{mmol}^{-1} \times \text{cm}^{-1}$, 267 nm)	100-110 mM ($\epsilon = 9.8 \text{ l} \times \text{mmol}^{-1} \times \text{cm}^{-1}$, 262 nm)
A_{250 nm} / A_{260 nm} (22 °C, pH 7.0)	0.78 ± 0.02	0.82 ± 0.02	1.15 ± 0.03	0.64 ± 0.02	0.74 ± 0.02
A_{280 nm} / A_{260 nm} (22 °C, pH 7.0)	0.15 ± 0.01	0.97 ± 0.02	0.67 ± 0.02	0.74 ± 0.02	0.38 ± 0.02
A_{290 nm} / A_{260 nm} (22 °C, pH 7.0)	NA	0.30 ± 0.02	0.28 ± 0.02	0.24 ± 0.02	0.04 ± 0.01
pH (4 °C)	8.5 ± 0.1	8.5 ± 0.1	8.5 ± 0.1	8.5 ± 0.1	8.5 ± 0.1
dNTP (HPLC area)	≥ 99.0w%	≥ 99.0%	≥ 99.0%	≥ 99.0%	≥ 99.0%
dNDP (HPLC area)	≤ 0.9%	≤ 0.9%	≤ 0.9%	≤ 0.9%	≤ 0.9%
dNMP (HPLC area)	≤ 0.5%	≤ 0.5%	≤ 0.5%	≤ 0.5%	≤ 0.5%
Low Copy Long Range PCR (18 kb, lambda DNA, template dilution series) ⁽²⁾	PCR fragment with 100 pg of template or less	PCR fragment with 100 pg of template or less	PCR fragment with 100 pg of template or less	PCR fragment with 100 pg of template or less	PCR fragment with 100 pg of template or less ⁽²⁾
RT-PCR (600 bp fragment, human GAPDH gene, template dilution series)	PCR fragment with 100 pg of template or less	PCR fragment with 100 pg of template or less	PCR fragment with 100 pg of template or less	PCR fragment with 100 pg of template or less	NA
Contamination with bacterial or human DNA	not detectable	not detectable	not detectable	not detectable	not detectable
DNases, RNases, Nicking Activity	not detectable	not detectable	not detectable	not detectable	not detectable
Proteases	not detectable	not detectable	not detectable	not detectable	not detectable

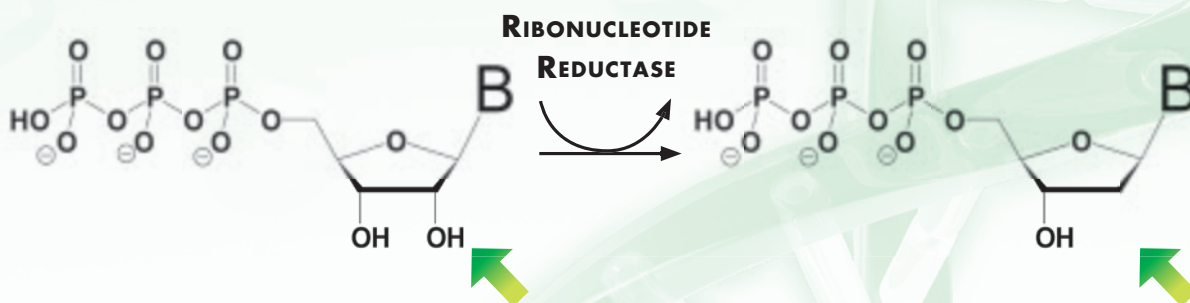
(1) Extinction coefficients: Cavaluzzi & Borer (2004) *Nucleic Acids Res.* **32(1)**:e13

(2) For dUTP: Low Copy PCR (1 kb, lambda DNA, template dilution series)



Production Technology

Jena Bioscience is one of only a few primary manufacturers of premium quality dNTPs for PCR. Our dNTPs are synthesized by experienced personnel in Germany using enzymatic & chemical technologies followed by chromatographic cascades. While dTTP is synthesized by chemical phosphorylation of thymidine, dATP, dCTP, dGTP, and dUTP are manufactured from their corresponding Ribo-NTPs in a single step employing the highly specific enzyme Ribonucleotide Reductase (Fig. 1). Jena Bioscience's annual manufacturing capacity is multiple kilograms per year corresponding to more than 100 L of 100 mM dNTP solutions.



B = ADENINE, CYTOSINE, GUANINE, URACIL

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Figure 1

The bacterial enzyme ribonucleotide reductase selectively reduces the 2'-OH-group of NTP raw materials resulting in their corresponding dNTPs. This enzymatic synthesis is performed on the kg-scale and reaches turnover of nearly 100% within a few days.

Quality

With increasing level of sophistication of PCR processes and regulatory requirements, highest dNTP quality with minimal batch-to-batch variations is simply inevitable. According to Jena Bioscience's DIN ISO 9001:2000 certified quality management system each dNTP lot is assayed by most stringent criteria regarding purity and functionality:

Purity

Purity of dNTPs is one important parameter for assessing their suitability for PCR reactions run by clinical/diagnostic laboratories or molecular biologists. Even minute amounts of impurities present in dNTP preparations may interfere with PCR, and appropriate technology for detecting and eliminating such impurities is pivotal. In principle, there are three types of impurities that need to be addressed:

I. Nucleosidic Contaminants: Include other dNTPs (e.g. deaminated/methylated dNTPs or dNTPs with a different base moiety), NTPs, and other deoxynucleoside phosphates such as dNMP, dNDP, or their tetra- and polyphosphates.

II. Inorganic Species: Include chemicals used during dNTP production/purification such as chloride, acetate, or pyrophosphate as well as contaminants potentially present in raw materials (e.g. heavy metals) or resulting from dNTP hydrolysis (phosphate).



III. Macromolecular Contaminants: These include nucleic acids (DNA or RNA) as well as traces of enzymatic activities (DNases, RNases, Proteases, and DNA nicking activities).

(I) Nucleosidic Contaminants – alongside other UV absorbing species – are detected by Reversed Phase HPLC (RP-HPLC). dNTP preparations showing RP-HPLC purity of $\geq 99\%$ (UV-absorbing contaminants $\leq 1\%$, respectively) are considered state of the art. Consistent and reproducible detection of impurities in the sub-percent range however, requires modern instrumentation as well as optimized methodology (Fig. 2). Only then reliable RP-HPLC purities of $\geq 99\%$ can be reported and guaranteed.

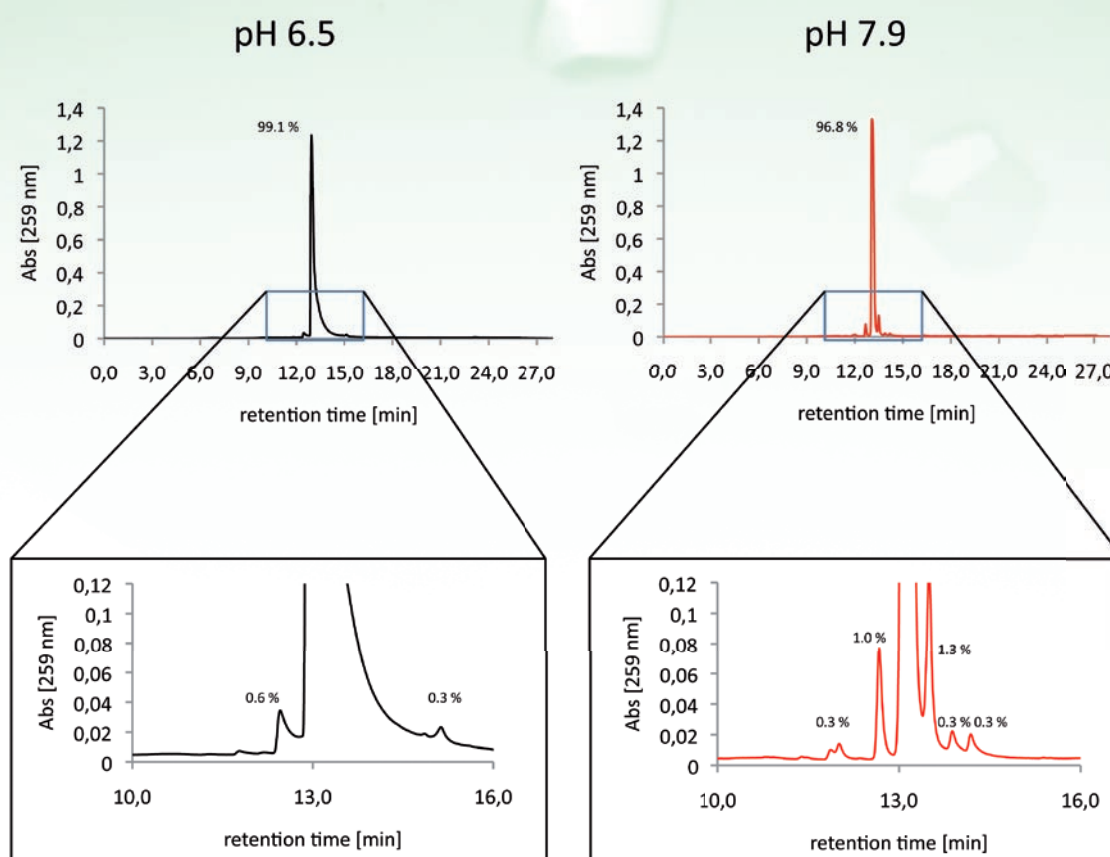


Figure 2

At Jena Bioscience a separate HPLC system (2008 Shimadzu Prominence with diode array detector SPD 20, Prontosil column 120-5-C18 250 \times 4.6 mm) is dedicated solely for detection of impurities in dNTP preparations. This system was used for analysis of dATP purchased from a competitor in Q3/2009 using 0.1 M TEAA buffer/acetonitrile for elution. Under standard conditions (pH 6.5) the apparent purity of the material is 99.1%. When adjusting buffer to pH 7.9 the resolution improves and the assay reveals that purity of dATP (96.8%) is clearly below specification. Major impurities were separated and analyzed by mass spectrometry and identified as dADP (1.0%) and Deoxyadenosine-tetraphosphate (1.3%).



(III) Inorganic Species: Presence of critical concentrations of inorganic species may result from contaminated raw materials and inadequate manufacturing processes. Since they interfere with PCR they are commonly termed “PCR inhibitors”. They do not absorb in the UV and would escape detection by RP-HPLC. Hence, Jena Bioscience uses Analytical Anion Chromatography, GC/FID, and ICP-MS for analyses (Fig. 3).

Species	Concentration reported to be critical in PCR	Maximum tolerated concentration in Jena Bioscience's dNTPs	
		in PCR mixture (0.2 mM = dilution 1:500)	in 100 mM dNTP solutions
Chloride Cl^-	25 mM	≤ 0.004 mM	≤ 2 mM
Acetate CH_3COO^-	5 mM	≤ 0.004 mM	≤ 2 mM
Phosphate PO_4^{3-}	no systematic data available	≤ 0.0004 mM	≤ 0.2 mM
Pyrophosphate $\text{P}_2\text{O}_6^{4-}$	0.1...0.3 mM	≤ 0.0001 mM	≤ 0.05 mM
Magnesium Mg^{2+}	1.5 mM ⁽¹⁾	≤ 0.0005 mM	≤ 0.25 mM
Calcium Ca^{2+}	1 mM	≤ 0.0005 mM	≤ 0.25 mM
Total Heavy Metals ⁽²⁾	no systematic data available	≤ 0.01 $\mu\text{g} \times \text{ml}^{-1}$	≤ 5 $\mu\text{g} \times \text{ml}^{-1}$

(1) Standard concentration in PCR

(2) Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sn, U

Figure 3

Jena Bioscience's maximum tolerated concentrations of common PCR inhibitors ensure their presence in a PCR reaction at levels several orders of magnitude below critical values. The species are determined with a 2006 Shimadzu Ion Chromatograph with a CDD 6A detector (chloride and phosphate), with a 2005 Shimadzu Gas Chromatograph 17A (acetate), a 2003 Thermo Fisher ICP-MS (all metals), and pyrophosphate is measured photometrically.

(III) Macromolecular Contaminants: The most critical macromolecules potentially present in dNTPs are human DNA and bacterial DNA. Enzymes of bacterial origin are commonly used during enzymatic dNTP synthesis, and human DNA is ubiquitously present during dNTP handling. Since the presence of only a few copies of such genomic DNA may result in false positive PCR, Jena Bioscience analyses each dNTP lot at the very end of the production process using qPCR (Fig. 4). Testing for residual enzymatic activities is performed with FRET-probes (DNases, RNases, Nicking activities) and a colorimetric UV-assay is used for detection of proteases.



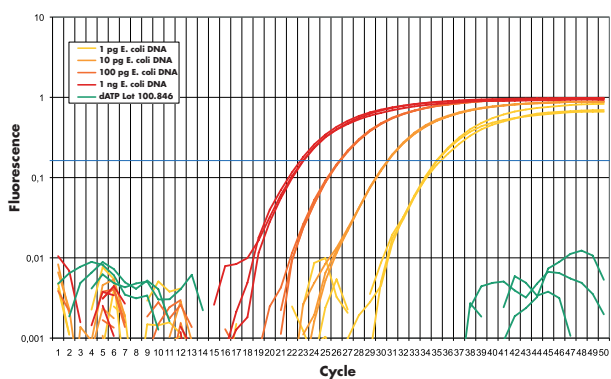
Deoxynucleotides (dNTPs)



8

BACTERIAL DNA

DETECTION OF BACTERIAL DNA



HUMAN DNA

DETECTION OF HUMAN DNA

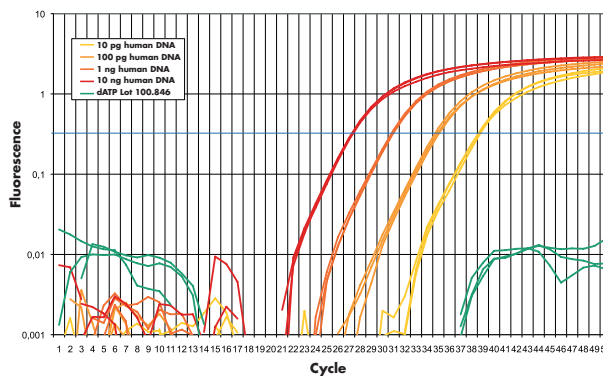


Figure 4

A multiplex qPCR assay verifies absence of contaminating bacterial or human DNA. Bacterial DNA is amplified using primers and probes for the 16S rRNA gene. Human DNA is detected by amplification of a beta-actin gene fragment. Traces of contaminating DNA are typically detected at *ct*-values in between 35 and 45.



Functionality

After ensuring premium purity of dNTPs at the end of their production process, samples of each lot are subsequently tested by a Low Copy Long Range PCR and a Reverse Transcription PCR. Both assays are performed with limiting amount of template which allows detection of even minute variations in dNTP performance. (Fig. 5 & 6)

Decreasing amount of template (pg)

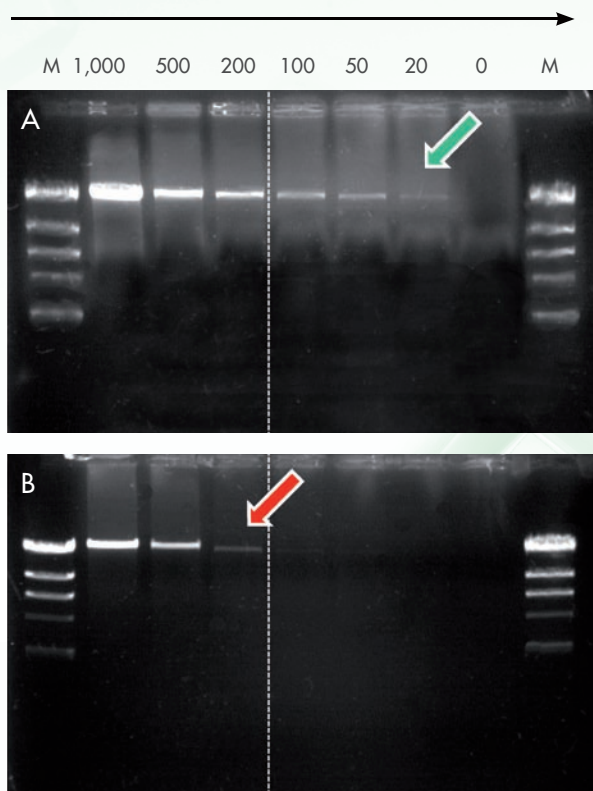


Figure 5

Each dNTP lot is assayed under defined conditions in an 18 kb PCR reaction with dilution of template. Amount of template starts at 1,000 pg and is reduced to zero (no template = negative control). Batches yielding a visible PCR product with 100 pg of template or less are accepted.

A: Conforming dNTP mix yielding a visible PCR product with 20 pg of template.

B: Non-conforming dNTP mix yielding a visible PCR product with 200 pg of template.

Decreasing amount of template (pg)

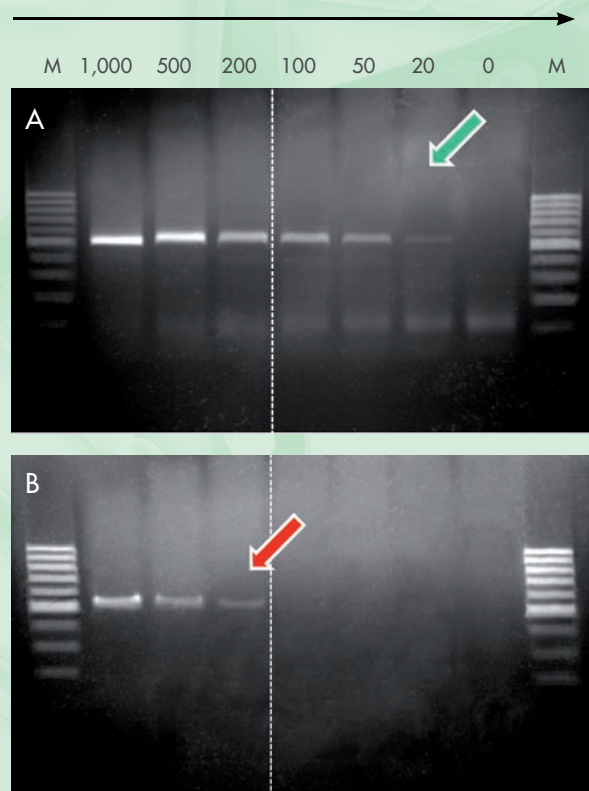


Figure 6

Since Reverse Transcription PCR (RT-PCR) is considered one of the most stringent assays for dNTP quality, Jena Bioscience assays each lot in a RT-PCR template dilution series. From decreasing amounts of human genomic RNA (1,000 pg to zero) a 600 bp fragment of the human GAPDH gene is amplified. dNTP lots yielding a visible RT-PCR product with 100 pg of template or less are accepted.

A: Conforming dNTP mix yielding a visible PCR product with 20 pg of template.

B: Non-conforming dNTP mix yielding a visible PCR product with 200 pg of template.



Terms and Conditions of Sales

Ordering

The following options are available for ordering products directly from Jena Bioscience:

- Mail orders
- Telephone orders
- 24 hour fax ordering
- Online ordering

Please provide the following information when ordering:

- Your name, name of institution
- Billing and shipping address
- PO number (if applicable)
- Catalog number of products and quantity needed
- Contact person and contact data for questions

Mail orders

Please send your mail orders to the following address:

Jena Bioscience GmbH
Loebstedter Strasse 80
07749 Jena, Germany

Telephone orders

We will accept telephone orders from Monday to Friday between 8:00 am and 16:00 pm Central European Time.

+49 – 3641 – 628 5000

24 hour fax ordering

Please send your fax order to:

+49 – 3641 – 628 5100

Online ordering

Jena Bioscience products can be ordered online. When ordering by e-mail, please direct your orders to: orders@jenabioscience.com

Products can also be ordered online through our online shop. Go to <http://www.jenabioscience.com> and follow the instructions.

Important Notice:

Products that have been ordered by mistake cannot be returned to Jena Bioscience. Products that are returned unrequestedly to Jena Bioscience will not be accepted, but fully charged to the customer's account.

Shipping

All customers will receive a fax confirmation of the order with invoice and shipping waybill number.

International orders are shipped either by General Overnight, by FedEx or by UPS Express service, depending on the customer's location and on the products to be shipped. Domestic shipments within Germany are sent by General Overnight Express service. If you wish your order to be shipped by a different carrier, please contact us and provide all necessary information with your order.

All orders are shipped EXW (Incoterms 2000). Please contact us if a different shipping term is required for your order.

Prices and Charges

Please note that the prices of products in the catalog and on our website do not include freight charges, duties, taxes or customs fees.

Freight charges will be prepaid and added to the invoice. Freight charges for online orders are indicated when you check out of the online store. If you need information on freight charges for your particular order, please contact us with all necessary information.

Jena Bioscience will not pay any duties, taxes or customs fees.

Products and prices are subject to change without notice. Current pricing will be confirmed at the time of your order. No minimum order required.

Payment

Invoices will be issued after your order has been shipped and will be sent to the billing address by separate mail. Invoices will not be included within the shipments. In case of partial deliveries, separate invoices will be issued after each shipment has left Jena Bioscience. You will find payment information (bank addresses and account data) on each invoice. Jena Bioscience accepts payment by:

Check

Please send your payment checks to the following address:

Jena Bioscience GmbH
Loebstedter Strasse 80
07749 Jena, Germany

We kindly ask you to make sure that our invoice number and your customer number appear on the cheque.

Wire transfer

Please remit your payments to one of the following bank accounts:

<i>Bayerische Hypo- und Vereinsbank AG</i>	<i>Account No.: 4196090</i>
<i>Niederlassung Thüringen</i>	<i>Bank code (BLZ): 83020087</i>
<i>Schillerstrasse 4</i>	<i>IBAN: DE 05830200870004196090</i>
<i>07745 Jena, Germany</i>	<i>SWIFT: HYVEDEMM463</i>
<i>Sparkasse Jena-Saale-Holzland</i>	<i>Account No.: 32417</i>
<i>Ludwig-Weimar-Gasse 5</i>	<i>Bank code (BLZ): 83053030</i>
<i>07743 Jena, Germany</i>	<i>IBAN: DE 22830530300000032417</i>

Credit card

Jena Bioscience accepts the following credit cards:

- VISA
- Mastercard
- American Express



If you wish to pay by credit card, please provide the following credit card information:

- Card holder
- Card number
- Expiry date
- Security code (VISA/Mastercard: 3 digits, to be found on your card's back side in the upper right corner of the signature field; AmEx: usually 4 digits, to be found on the front side of your card above the card number)

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