BIOTOOLS B&M LABS.S.A.

*Manufactured by:*

BIOTOOLS B&M Labs, S.A.
Valle de Tobalina - 52 - Nave 39
28021 Madrid
Spain

Tel. (34) 91 710 00 74
Fax (34) 91 505 31 18
e-mail: info@biotools.eu
www.biotools.eu

# SPEEDTOOLS PCR CLEAN-UP KIT

***Designed for the direct purification of DNA from PCR products or from agarose gels (TAE/TBE)***

# Instructions for Use

**(Cat. No. 21.200M/1/2)**

***PLEASE READ THE INSTRUCTIONS FOR USE THOROUGHLY BEFORE USING THE KIT, ESPECIALLY IF YOU ARE NOT FAMILIAR WITH THE PROTOCOL.***

# 1. BASIC PRINCIPLE

**SPEEDTOOLS PCR CLEAN-UP KIT** is designed for a rapid and easy isolation of DNA fragments from PCR reactions and TAE/TBE agarose gels.

DNA from PCR reactions or agarose gels binds to the silica membrane of the BINDING COLUMN in the presence of chaotropic salts from the binding BUFFER B. Contaminants like salts and soluble macromolecular components are removed by a simple washing step with ethanolic BUFFER T3. Pure DNA is finally eluted under low ionic strength conditions with slightly alkaline BUFFER E or sterile bidistilled water.

**Table 1. General Characteristics of the Kit**

| | |
|---|---|
| Elution volume | 15-50 µl |
| Binding Capacity | 15 µg |
| Yield | 85-95% |
| Time / Prep | 10 min/ 6 preps |

# 2. KIT SPECIFICATIONS

- The Kit is designed for the purification of DNA from TAE/TBE agarose gels and for the direct purification of PCR products.
- Buffer formulation ensures complete **removal of primer** from PCR reaction while **small DNA fragments are** still bound and **purified with high recovery**.
- DNA fragments from PCR reaction buffers rich in various detergents can be purified with high recovery.
- The adsorption of DNA to the column membrane is pH dependent. Optimal recovery is achieved by using TAE standard gels or reaction mixtures with pH 6-8.
- Standard as well as low melting agarose gels can be used.
- The obtained DNA fragments can be used directly in applications like automated fluorescent DNA sequencing, PCR, or any kind of enzymatic manipulation.

**Table 2. Intended Use of the Kit**

| | | |
|---|---|---|
| DNA fragments from agarose gels (standard and low melting) | YES | Section 7 |
| Concentration, removal of salts, enzymes, nucleotides and/or labelling reagents like biotin or radioactive ATP, etc | YES | Section 9 |
| Direct purification of amplified DNA | YES | Section 8 |
| Purification of reaction mixtures without or with SDS | YES | Section 9 |
| Purification of single stranded DNA | YES | Section 9 |
| Removal of small DNA fragments and primer-dimers | YES | Section 5 |

## 3. KIT CONTENTS

| SPEEDTOOLS PCR CLEAN-UP KIT | | | |
|---|---|---|---|
| | 10 Preps<br>Cat. No. 21.200M | 50 Preps<br>Cat. No. 21.201 | 250 Preps<br>Cat. No. 21.202 |
| BUFFER B<br>Binding Buffer | 10 ml | 2 x 25 ml | 5X (2 x 25 ml) |
| BUFFER T3 (concentrated)<br>Wash Buffer | 6 ml | 2 x 6 ml | 5X (2 x 6 ml) |
| BUFFER E<br>Elution Buffer | 5 ml | 15 ml | 5X 15 ml |
| BINDING COLUMNS | 10 ml | 50 ml | 5X 50 ml |
| 2 ml COLLECTION TUBES | 10 ml | 50 ml | 5X 50 ml |
| PROTOCOL | 1 | 1 | 1 |

## 4. ELUTION PROCEDURES

For the elution of DNA, one of the following solutions can be used: BUFFER E, included in the kit (5 mM Tris/HCl, pH 8.5), TE buffer, pH 8.5 or sterile bidistilled water, pH 8.5.

**NOTE:** EDTA in TE buffer may cause problems in subsequent reactions. The pH of the water should be checked before use to avoid lower recovery yields.

Table 3 shows the correlation between dispensed elution buffer volumes and typical recoveries for the purification of 1-5 µg of PCR fragments (for gel extraction, recovery is approx. 10% lower).

**Table 3. DNA Recovery with SPEEDTOOLS PCR CLEAN-UP Kit**

| FRAGMENT LENGTH | ELUTION VOLUME | RECOVERY |
|---|---|---|
| 65 bp | 15 µl<br>25 µl<br>50-100 µl | 85%<br>90%<br>95% |
| 400 bp | 15 µl<br>25 µl<br>50-100 µl | 85%<br>90%<br>95% |
| 700 bp | 15 µl<br>25 µl<br>50-100 µl | 85%<br>90%<br>95% |
| 1500 bp | 15 µl<br>25 µl<br>50-100 µl | 85%<br>90%<br>95% |

With an elution volume of 15 µl of BUFFER E, a typical recovery of 70-95% is usually obtained for DNA fragments between 50-10,000 bp resulting in highly concentrated eluates. If larger amounts (5-15 µg) of DNA have to be purified (e.g. from PCR reactions > 100 µl or gel slices > 200 mg), elution with at least 50 µl of BUFFER E is recommended. Primers are not bound to the membrane column.

Yields of larger fragments (> 5-10 Kbp) can be increased by using pre-warmed elution buffer (70°C): For elution, add pre-warmed BUFFER E and incubate for 1-2 min before collecting eluate by centrifugation.

## 5. REMOVAL OF SMALL DNA FRAGMENTS AND PRIMER-DIMERS

**SPEEDTOOLS PCR CLEAN-UP KIT** is designed to remove even traces of unused primers, and at the same time, to purify PCR products down to 65 bp. However, in some cases it is necessary to exclude these small fragments, e.g. primer-dimers or side products resulting from unspecific annealing, since they might interfere with your downstream sequencing or cloning applications.

Removal of double stranded DNA >65 bp can be achieved by diluting an aliquot of BUFFER B with sterile bidistilled water in an appropriate ration and then proceeding with the standard protocol. Diluting BUFFER B in a certain range lowers the binding efficiency for small fragments without compromising the recovery of larger PCR products. The dilution ratio to choose depends on the fragment size that is to be purified as well as on the PCR buffer system that is used. The smaller the fragment in question, the less you have to dilute BUFFER B.

**Figure 1. Purification of PCR reactions using BUFFER B dilutions.** ***Lane 1:*** *100 bp Ladder Marker (BIOTOOLS Cat. No. 31.006) -* ***Lane 2:*** *Sample (21, 50, 65, 79, 100, 164, 359, 645 and 982 bp fragments) -* ***Lane 3:*** *Purification with 100% BUFFER B -* ***Lane 4-12****: Purification using diluted BUFFER B with 1-9 volumes of sterile bidistilled water.*

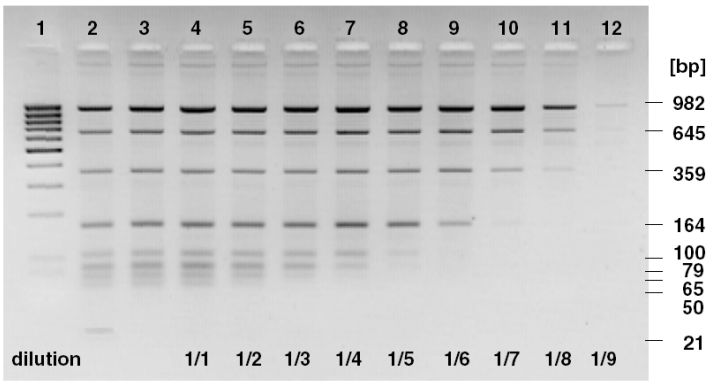



*Figure 1 shows a purification result with dilution series of BUFFER B. Non diluted BUFFER B (lane 3), as well as BUFFER B plus one volume of water (lane 4), lead to 100% recovery of a PCR fragments (lane 2). Use of more diluted BUFFER B cuts off more and more of the low molecular mass bands. Usually a dilution with 5 volumes of sterile bidistilled water should be sufficient to eliminate even larger unwanted primer-dimer fragments while purifying the 164 bp fragment with >90%.*

The influence of the PCR buffer system on the removal of small fragments is more complex. Some reaction buffers contain **detergents like Tween** or **additives like betaine** which lower the melting temperature of the DNA template. These substances are usually found in PCR buffers for high fidelity or long range PCR, they tend to **lower the binding efficiency of DNA to the silica membrane** and therefore have to be considered when choosing a dilution ratio of BUFFER B.

***As a rule of thumb if a PCR buffer system without special additives is used, adding 3 to 5 volumes of water to 1 volume of BUFFER B will lead to removal of small fragments up to 100 bp. Otherwise adding 1 to 3 volumes of sterile bidistilled water to 1 volume of BUFFER B will be sufficient.***

Therefore for each size of small fragments >65 bp that has to be removed, and for each PCR system, determine in advance the appropriate ratio of BUFFER B dilution.

## 6. PREPARATION OF WORKING SOLUTIONS AND STORAGE CONDITIONS

All kit components can be stored at room temperature (20-25°C) and are stable up to one year.

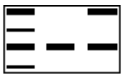
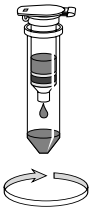
**NOTE:** BUFFER B contains chaotropic salt. Wear gloves and goggles.

Before starting any protocol with **SPEEDTOOLS PCR CLEAN-UP KIT** prepare BUFFER T3 as follow:

- Add 24 ml of 96-100% ethanol to each bottle of concentrated BUFFER T3

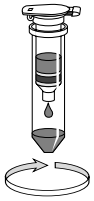
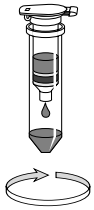
# 7. PROTOCOL FOR DNA EXTRACTION AND PURIFICATION FROM AGAROSE GELS

Before starting with the preparation, set incubator or water bath to 50°C. Prepare BUFFER T3 (see Section 6). Equilibrate the necessary amount of BUFFER E to 70°C.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **EXCISE DNA FRAGMENT**<br>*Take a clean scalpel to excise the DNA fragment from an agarose gel. Excise gel slice containing the fragment carefully to minimize the gel volume. Determine the weight of the gel slice and transfer it to a clean tube.* |  | Excise Gel Band |
| 2 | **GEL LYSIS**<br>For each 100 mg of agarose gel add 200 µl BUFFER B.<br>*For gels containing > 2% agarose, double the volume of BUFFER B. The maximum amount of gel slice per COLUMN is 400 mg or 200 mg of high percentage gel > 2%, in this case two loading steps are required.*<br>Incubate sample at 50ºC until the gel slices are dissolved (5-10 min). Vortex briefly every 2-3 min until the gel slices are completely dissolved |  | +<br>200 µl<br>BUFFER B<br>per<br>100 mg gel<br><br>50ºC,<br>5-10 min |
| 3 | **BIND DNA**<br>For each preparation, take one BINDING COLUMN and placed it into a 2 ml COLLECTION TUBE. Load the sample carefully.<br>Centrifuge 1 min at 11,000 × *g*. Discard flow-through and place the COLUMN back into the COLLECTION TUBE. |  | Load sample<br>into a column<br><br>*1 min,*<br>*11,000 × g* |
| 4 | **WASH SILICA MEMBRANE**<br>Add 600 µl BUFFER T3. Centrifuge 1 min at 11,000 x *g*. Discard flow-through and place the COLUMN back into the COLLECTION TUBE |  | +<br>600 µl<br>BUFFER T3<br><br>*1 min,*<br>*11,000 × g* |
| 5 | **DRY SILICA MEMBRANE**<br>Centrifuge 2 min at 11,000 x *g* to remove BUFFER T3 quantitatively (the column must not come in contact with the flow through).<br>*Residual ethanol from the buffer might inhibit subsequent reactions, therefore make sure that ethanol is completely removed. Total removal can be achieved by incubation of the Column for 2-5 min at 70ºC prior to elution step.* |  | *2 min,*<br>*11,000 × g* |
| 6 | **ELUTE PURE DNA**<br>Place the COLUMN into a new 1.5 ml microcentrifuge tube. Add 15-50 µl elution BUFFER E. Dispense buffer directly onto the silica membrane. Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x *g*. The eluate contains your pure DNA sample.<br>*Yield of larger fragmentes (> 5-10 Kbp) can be increased by using prewarmed elution buffer (70ºC).* |  | **+**<br>15-50 µl<br>BUFFER E<br>(70ºC)<br><br>Incubate RT<br>1 min<br><br>*1 min,*<br>*11,000 × g* |

# 8. PROTOCOL FOR DIRECT PURIFICATION OF PCR PRODUCTS

Before starting with the preparation, set incubator or water bath to 50°C. Prepare BUFFER T3 (see section 6). Equilibrate the necessary amount of BUFFER E to 70°C.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **ADJUST DNA BINDING CONDITIONS**<br>Mix 1 volume of sample with 2 volumes of BUFFER B.<br>*For sample volumes < 100 µl adjust the volume of the reaction mix to 100 µl using Buffer B or water*<br>For removal of DNA fragments > 65 bp, dilutions of BUFFER B can be used instead of 100% BUFFER B (see section 4). |  | +<br>2 VOL<br>BUFFER B<br>per<br>1 VOL<br>SAMPLE |
| 2 | **BIND DNA**<br>For each preparation, take one BINDING COLUMN and placed it into a 2 ml COLLECTION TUBE. Load the sample carefully.<br>Centrifuge 1 min at 11,000 × *g*. Discard flow-through and place the COLUMN back into the COLLECTION TUBE. |  | Load sample into a column<br>*1 min,*<br>*11,000 × g* |
| 3 | **WASH SILICA MEMBRANE**<br>Add 600 µl BUFFER T3. Centrifuge 1 min at 11,000 x *g*. Discard flow-through and place the COLUMN back into the COLLECTION TUBE. |  | +<br>600 µl<br>BUFFER T3<br>*1 min,*<br>*11,000 × g* |
| 4 | **DRY SILICA MEMBRANE**<br>Centrifuge 2 min at 11,000 x *g* to remove BUFFER T3 quantitatively (the column must not come in contact with the flow through).<br>*Residual ethanol from the buffer might inhibit subsequent reactions, therefore make sure that ethanol is completely removed. Total removal can be achieved by incubation of the Column for 2-5 min at 70ºC prior to elution step.* |  | *2 min,*<br>*11,000 × g* |
| 5 | **ELUTE PURE DNA**<br>Place the COLUMN into a new 1.5 ml microcentrifuge tube. Add 15-50 µl elution BUFFER E. Dispense buffer directly onto the silica membrane. Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x *g*. The eluate contains your pure DNA sample.<br>*Yield of larger fragments (> 5-10 Kb) can be increased by using prewarmed elution buffer (70ºC).* |  | **+**<br>15-50 µl<br>BUFFER E<br>(70ºC)<br>Incubate<br>1 min<br>*1 min,*<br>*11,000 × g* |

## 9. SUPPORT PROTOCOLS

### A) Concentration and removal of salts, enzymes, etc. Purification of samples without SDS (BUFFER B included in the Kit)

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **ADJUST DNA BINDING CONDITIONS**<br>Mix 1 volume of sample with 2 volumes of BUFFER B.<br>*If the sample contains large amounts of detergents or other critical substances, double the volume of BUFFER B.* |  | +<br>2 VOL<br>BUFFER B<br>per<br>1 VOL<br>SAMPLE |
| 2 | **Proceed with Step 2 of the *Protocol for direct purification of PCR products* (Section 8)** | | |

### B) Concentration and removal of salts, enzymes, etc. Purification of samples containing SDS (BUFFER BS Cat. No. 21.203[1] not included)

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **ADJUST DNA BINDING CONDITIONS**<br>Mix 1 volume of sample with 5 volumes of BUFFER BS. |  | +<br>5 VOL<br>BUFFER BS<br>per<br>1 VOL<br>SAMPLE |
| 2 | **Proceed with Step 2 of the *Protocol for direct purification of PCR products* (Section 8)** | | |

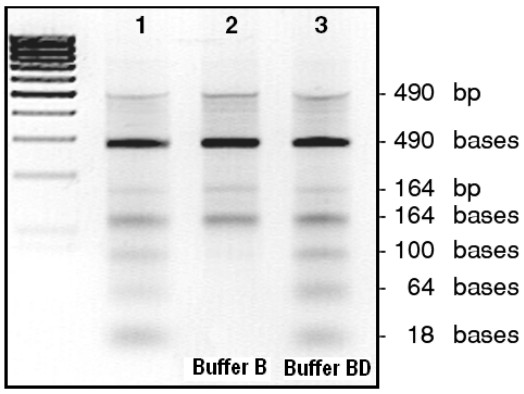
### C) Purification of single stranded DNA (BUFFER BD Cat. No. 21.204[2] not included)

BUFFER B (included in the Kit) is able to bind single stranded DNA (ssDNA) > 150 bases. Shorter oligonucleotides, especially primers, are completely removed. For removing short ssDNA the additional BUFFER BD can be used.

| STEP | DESCRIPTION | | |
|---|---|---|---|
| 1 | **ADJUST DNA BINDING CONDITIONS**<br>Mix 1 volume of sample with 2 volumes of BUFFER BD.<br>*If the sample contains large amounts of detergents or other critical substances, double the volume of BUFFER BD.* |  | +<br>2 VOL<br>BUFFER BD<br>per<br>1 VOL<br>SAMPLE |
| 2 | **Proceed with Step 2 of the *Protocol for direct purification of PCR products* (Section 8)** | | |

[1] BUFFER BS has to be ordered separately Cat. No. 21.203
[2] BUFFER BD has to be ordered separately Cat. No. 21.204

**Figure 2. Purification of dsDNA and ssDNA using BUFFER B and BUFFER BD.** PCR fragments, amplified using one phosphorylated and one dephosphorylated primer, were partially digested with λ-Exonuclease. Samples were purified using BUFFER B and BUFFER BD and run on a 1 % TAE agarose gel. Remaining double stranded DNA can be seen as faint bands. The corresponding ssDNA is running slightly faster due to secondary structure formation. Compared to the input DNA (lane 1), BUFFER B removes ssDNA < 150 bases (lane 2), whereas BUFFER BD leads to full recovery of even primer oligonucleotides (lane 3).




## 10. TROUBLESHOOTING

| Problem | Possible cause and suggestions |
|---|---|
| Low DNA yield | ***Reagents not applied properly***<br>• Add indicated volume of 96-100% ethanol to BUFFER T3 concentrate and mix well before use.<br>***Incomplete dissolved gel slice***<br>• Increase time or add another two volumes of BUFFER B and vortex the tube every 2 minutes during incubation at 50ºC. Small pieces of gel are hardly visible and contain DNA that will be lost for purification.<br>***Insufficient drying of the COLUMN membrane***<br>• Centrifuge 5 min at 11,000 x *g* or incubate column for 2-5 min at 70ºC before elution to remove ethanolic BUFFER T3 completely. Ethanolic contaminations are also indicated by gel-loading problems (samples float out of gel slots). Remove the column carefully from centrifuge and collection tube and avoid contact of the column with flow-through.<br>***Not enough elution buffer***<br>• Especially when larger amounts of DNA (> 5 µg) are bound, increase elution buffer volume up to 100 µl.<br>***Isolation of large DNA fragments***<br>• Preheat elution BUFFER E to 70ºC, and incubate on the silica membrane at room temperature for 2 min before centrifugation. |

| Problem | Possible cause and suggestions |
|---|---|
| Incomplete lysis of agarose slices | ***High amount of agarose***<br>• Use doubled volumes of BUFFER B for highly concentrated and/or low melting point agarose gels.<br>***Insufficient time and temperature***<br>• Check incubation temperature. Depending on the weight of the gel slice, incubation can be prolonge up to 20 min. Vortex every 2 min and check integrity of the gel slice. Very large gel slices can be quenched or crushed before addition of BUFFER B. |
| Suboptimal performance of DNA in NanoDrop® Spectophotometer Analysis | ***Carry-over of traces of silica particles***<br>• NanoDrop® Spectrophotometer technology is very sensitive to any particles included in the sample material. To pellet the silica particles centrifuge > 2min at 11,000 x g and take the supernatant for further use. |
| Suboptimal performance of DNA in sequencing, restriction or ligation reactions | ***Carry-over of ethanol/ethanolic BUFFER T3***<br>• Centrifuge 5 min at 11,000 x *g* or better incubate the column for 5-10 min at 70ºC before elution to remove ethanolic BUFFER T3 completely. Ethanolic contaminations are also indicated by gel loading problems (samples float out of gel slots). Remove the column carefully from the centrifuge and collection tube, and avoid contact of the column with the flow-through.<br>***Carry-over of chaotropic salts***<br>• Perform a second washing step with BUFFER T3 in case of sensitive downstream applications to remove last traces of BUFFER B.<br>***Elution of DNA with buffers other than BUFFER E e.g. TE Buffer (Tris/EDTA)***<br>• EDTA might inhibit sequencing reactions. In this case it is recommended to re-purify DNA and elute in BUFFER E or sterile bidistilled water.<br>***Not enough DNA used for sequencing reaction***<br>• Quantify DNA by agarose gel electrophoresis before setting up sequencing reactions. |

## 11. SAFETY INSTRUCTIONS

The following components of the SPEEDTOOLS PCR CLEAN-UP KIT contains hazardous contents. Wear gloves and goggles and follow the safety instructions given in this section.

| Reagent | Hazard Contents | Hazard Symbol | Risk Phrases | Safety Phrases |
|---|---|---|---|---|
| BUFFER B | Guanidine thiocyanate | Xn* Harmful by inhalation, in contact with the skin and if swallowed<br>Keep away from food, drink and animal feedstuffs | R 20/21/22 | S 13 |

* Label not necessary, if quantity below 125 g or ml (concerning 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 42 and TRGS 200 7.1)

## 12. ORDERING INFORMATION

| PRODUCT | BIOTOOLS Cat. No. | Content |
|---|---|---|
| BUFFER BS | 21.203 | 150 ml |
| BUFFER BD | 21.204 | 100 ml |

## 13. PRODUCT USE RESTRICTION AND WARRANTY

1. Product for research purposes and *in vitro uses* only.
2. No claim or representation is intended for its use to identify any specific microorganism or for clinical use (diagnostic, prognosis, therapeutic, or blood bank).
3. It is rather the responsibility of the user to verify the use of the kit for a specific application range as the performance characteristic of this kit has not been verified for a specific microorganism.
4. The kit includes documentation stating specifications and other technical information. Follow the instructions in order to obtain highly pure DNA fragments.
5. BIOTOOLS warrants meeting the stated specifications of the kit. Any product not fulfilling the specifications included in the product sheet will be replaced.
6. BIOTOOLS obligation and purchaser's rights under this warranty are limited to the replacement by BIOTOOLS of any product that is shown defective in fabrication, and that must be returned to BIOTOOLS, freight prepaid, or at BIOTOOLS' option, replacement of the purchasing price.
7. BIOTOOLS does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded). Any complaint on damaged goods during transport must be directed to the handling or transport agent.
8. BIOTOOLS has no responsibility for damages, whether direct or indirect, incidental or consequential of improper or abnormal use of this product. Nor has any responsibility for defects in products or components not manufactured by BIOTOOLS, or against damages resulting from such non-BIOTOOLS components or products.
9. BIOTOOLS makes no other warranty of any kind whatsoever, and specifically disclaims and excludes all other warranties of any kind or nature whatsoever, directly or indirectly, express or implied, including, without limitation, as to the suitability, reproductively, durability, fitness for a particular purpose or use, merchantability, condition, or any other matter with respect to BIOTOOLS products.
10. The warranty provided herein and the data, specifications and descriptions of this kit appearing in BIOTOOLS published catalogues and product literature are BIOTOOLS sole representations concerning the product and warranty. No other statements or representations, written or oral, by BIOTOOLS employees, agent or representatives, except written statements signed by a duly authorized officer of BIOTOOLS are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.
11. Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information. You may also contact your local distributor for general scientific information.
12. Applications mentioned in BIOTOOLS literature are provided for informational purposes only. BIOTOOLS does not warrant that all applications have been tested in BIOTOOLS laboratories using BIOTOOLS products. BIOTOOLS does not warrant the correctness of any of those applications. For more information contact our Technical Dept (info@biotools.eu).

**Manufactured by:**

BIOTOOLS, Biotechnological & Medical Laboratories, S.A. have been evaluated and certified to accomplish ISO 9001:2000 requirements for the following activities: Research and development of biotechnology products and manufacture of biotechnology and in vitro products.
Valle de Tobalina – 52 – Nave 39, 28021 Madrid – España