For life science research only.
Not for use in diagnostic procedures.

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# High Pure PCR Product Purification Kit

**Version: 19**
Content version: March 2018

For purification of PCR reaction products

| | |
|---|---|
| **Cat. No. 11 732 668 001** | 1 kit<br>up to 50 purifications |
| **Cat. No. 11 732 676 001** | 1 kit<br>up to 250 purifications |

**Store the kit at +15 to +25°C.**

# 1. General Information

## 1.1. Contents

*Both pack sizes of the kit contain the same components; they differ only in the amount of components.*

| Vial / Bottle | Cap | Label | Function / Description | Catalog Number | Content |
|---|---|---|---|---|---|
| 1 | green | Binding Buffer | 3 M guanidine-thiocyanate, 10 mM Tris-HCl, 5% ethanol (v/v), pH 6.6 (+25°C) | 11 732 668 001 | 30 ml |
| | | | | 11 732 676 001 | 150 ml |
| 2 | blue | Washing Buffer | 20 mM NaCl, 2 mM Tris-HCl, pH 7.5 (+25°C) (final concentrations after addition of ethanol) | 11 732 668 001 | 10 ml, add 40 ml absolute ethanol |
| | | | | 11 732 676 001 | 50 ml, add 200 ml absolute ethanol |
| 3 | colorless | Elution Buffer | 10 mM Tris-HCl, pH 8.5 (25°C) | 11 732 668 001 | 40 ml |
| | | | | 11 732 676 001 | 40 ml |
| 4 | | High Pure Filter Tubes | | 11 732 668 001 | One bag with 50 polypropylene tubes with two layers of glass fiber fleece, for processing up to 700 µl sample volume. |
| | | | | 11 732 676 001 | Five bags with 50 polypropylene tubes with two layers of glass fiber fleece, for processing up to 700 µl sample volume. |
| 5 | | Collection Tubes | | 11 732 668 001 | One bag with 50 polypropylene tubes (2 ml). |
| | | | | 11 732 676 001 | Five bags with 50 polypropylene tubes (2 ml). |

⚠ ***All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a 37°C water bath until the precipitates have dissolved.***

*The buffers can show a slight yellow color. This will have no impact on the function of the buffer*

## 1.2. Storage and Stability

### Storage Conditions (Product)

Kit components are stable at +15 to +25°C until the expiration date printed on the label.

⚠ ***Improper storage of the kit at +2 to +8°C (refrigerator) or −15 to −25°C (freezer) may lead to formation of salt precipitates in the buffers which will adversely affect plasmid DNA purification.***

### Storage Conditions (Working Solution)

| Solution | Storage |
|---|---|
| Wash Buffer | +15 to +25°C |

## 1.3. Additional Equipment and Reagents Required

- Absolute ethanol
- Agarose*
- TAE buffer (40 mM Tris-acetate, 1 mM EDTA), pH 8.0 or
- TBE buffer (89 mM Tris-borate, 2 mM EDTA), pH 7.8
- Electrophoresis equipment
- Sterile scalpel
- Isopropanol
- Standard tabletop microcentrifuge capable of 13,000 x g centrifugal force
- 1.5 ml, sterile microcentrifuge tubes

## 1.4. Application

The kit is designed for the efficient and convenient isolation of PCR products from amplification reactions. Primers, mineral oil, salts, unincorporated nucleotides, and the thermostable DNA polymerase may inhibit subsequent enzymatic reactions (*e.g.*, labeling, sequencing or cloning of the PCR products). This kit is also recommended for the purification of cDNA (Footitt, S. et al. (2003)).
In addition, nucleic acids from other modification reactions *(e.g.,* restriction - endonuclease digests, alkaline-phosphatase treatment, or kinase reactions) can be purified using this kit. It can also be applied to concentrate dilute nucleic-acid solutions.

## 1.5. Preparation Time

| | |
|---|---|
| Total time | Approximately 10 minutes |

# 2. How to Use this Product

## 2.1. Before you Begin

### Sample Materials

Samples should contain:
- Solution volume up to 100 µl
- Amplified DNA products that are at least 100 bp and less than 50 kb
- Modified DNA (*e.g.,* DNA processed by restriction enzyme (Löbner, K. et al. (2002)), alkaline phosphatase treatment, and kinase or other enzymatic reactions (Chang, PC et al. (2001); Salesse, S. et al. (2003)) that is at least 100 bp and less than 50 kb.
- Hapten-labeled DNA (*e.g.,* DIG-labeled)
- RNA from *in vitro* transcription reactions in order to remove radioactive or non-radioactive unincorporated label from transcribed RNA.
- First strand cDNA (Footitt, S. et al.(2003))

*The High Pure PCR Product Purification Kit may also be used to prepare DNA from a 100 mg agarose gel slice, see Protocol Purification of DNA Fragments from Agarose Gel and (D'Errico, I. et. al, (2005); Falchetti, A. et al. (2005))*

### General Considerations

#### Handling Requirements

⚠ ***Binding Buffer contains guanidine hydrochloride which is an irritant. Always wear gloves and follow standard safety precautions to minimize contact when handling.***
⚠ ***Never store or use the Binding Buffer and Inhibitor Removal Buffer near human or animal food.***
⚠ ***Avoid contact of the Binding Buffer and Inhibitor Removal Buffer with the skin, eyes, or mucous membranes. If contact does occur, immediately wash the affected area with large amount of water. Burns can occur if left untreated. If the reagent spills, dilute with water before wiping dry.***
⚠ ***Do not use any modified ethanol.***
⚠ ***Do not pool reagents from different lots or from different bottles of the same lot.***
⚠ ***Immediately after usage, close all bottles in order to avoid leakage, varying buffer concentrations or buffer conditions. After first opening store all bottles in an upright position.***
⚠ ***Do not allow the Binding Buffer to mix with sodium hypchlorite found in commercial bleach solutions. This mixture can produce a highly toxic gas.***

# Safety Information

## Laboratory procedures

- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles. Use the general precautions described in the literature.
- Wash hands thoroughly after handling samples and reagents.
- Finish each phase of the PCR/RT-PCR workflow before proceeding to the next phase. For example, you should finish PCR/RT-PCR sample preparation before starting PCR/RT-PCR set-up. Sample preparation, PCR/RT-PCR set-up and the PCR/RT-PCR run itself should also be performed in separate locations.

## Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.

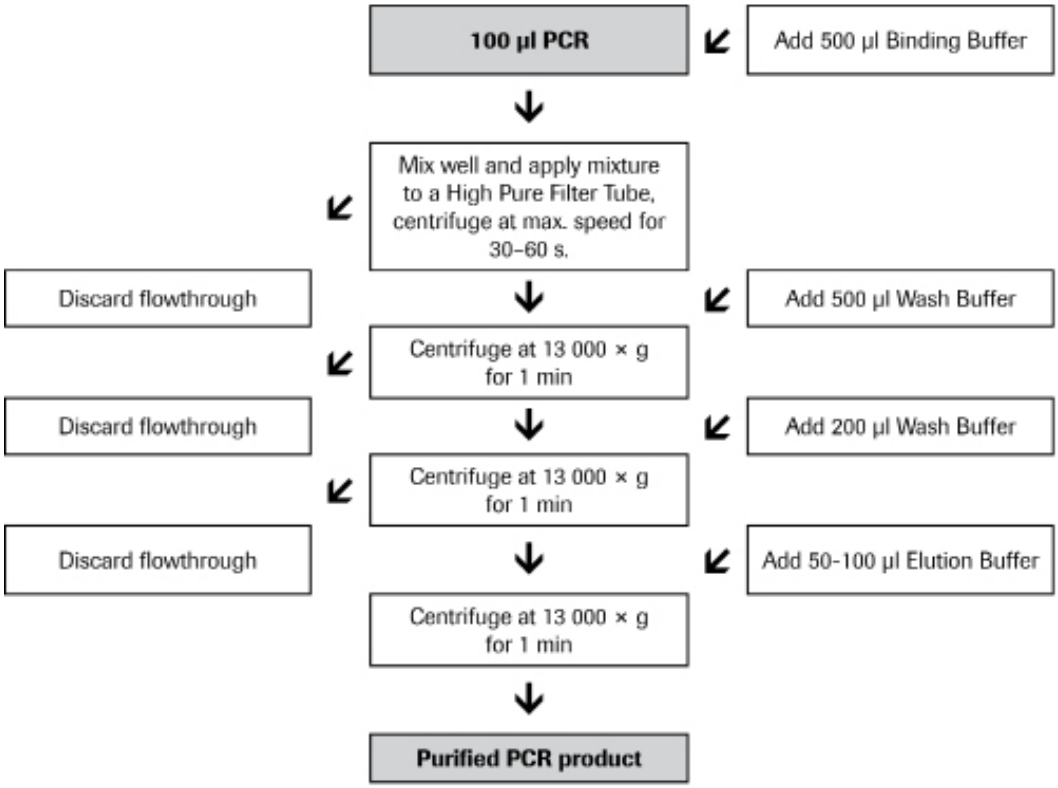
# Working Solution

Beside the ready-to-use solutions supplied with this kit, you will need to prepare the following working solution:

| Content | Reconstitution / Preparation | Storage / Stability | For use in... |
|---|---|---|---|
| Wash Buffer (Vial 2; blue cap) | Add 40 ml (200 ml) absolute ethanol to Wash Buffer.<br>*Label and date bottle accordingly after adding ethanol.* | ▪ Store at +15 to +25°C.<br>▪ Stable until the expiration date printed on kit label | PCR product purification: removal of nucleotides, primers, salts and proteins. |

# 2.2. Protocols

## Experimental overview




## Purification of PCR Products in Solution after Amplification

In the following protocol the purification of PCR products in solution after amplification is described.

*i To process a larger sample (>100 µl), either increase proportionally the amount of Binding Buffer (Step 1), or divide the larger sample into several 100 µl aliquots and process each as a separate sample.*

1. After PCR is finished, adjust total volume for each PCR tube (reaction components + DNA product) to 100 µl:
   – Add 500 µl Binding Buffer to each 100µl PCR tube.

   *i Mineral oil or wax does not need to be removed from the PCR solution before adding the Binding Buffer.*
   – Mix sample (Binding Buffer + PCR solution) well.

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2. – Insert one High Pure Filter Tube into one Collection Tube.
   – Transfer the sample from step 1 using a pipette to the upper reservoir of the Filter Tube.
   – Centrifuge 30 – 60 s at maximum speed in a standard table top centrifuge at +15 to +25°C.

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3. – Disconnect the Filter Tube, and discard the flowthrough solution.
   – Reconnect the Filter Tube to the same Collection Tube.

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4. Add 500 µl Wash Buffer to the upper reservoir.
   – Centrifuge 1 min at maximum speed (as above).

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5 – Discard the flowthrough solution.
– Recombine the Filter Tube with the same Collection Tube.
– Add 200 µl Wash Buffer.
– Centrifuge 1 min at maximum speed (as above).

*This second 200 µl wash step ensures optimal purity and full removal of Wash Buffer from the glass fibers.*

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6 – Discard the flowthrough solution and Collection Tube.
– Reconnect the Filter Tube to a clean 1.5 ml microcentrifuge tube

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7 – Add 50 – 100 µl Elution Buffer to the upper reservoir of the Filter Tube.
– Centrifuge 1 min at maximum speed.

⚠ ***Do not use water for elution since alkaline pH is required for optimal yield.***

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8 The microcentrifuge tube now contains the purified DNA.

⚠ ***When subsequent $OD_{260}$ determination is planned, centrifuge the eluate for more than 1 min at maximum speed to remove residual glass fibers from the eluate, because they may disturb absorbance measurement. Use an aliquot of the supernatant to determine concentration.***

*Either use the eluted DNA directly or store the eluted DNA at +2 to +8°C or −15 to −25°C for later analysis.*

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## Purification of DNA Fragments from Agarose Gel

In the following table the purification procedure for DNA from a 100 mg agarose gel slice is described:

1 Isolate DNA band of interest electrophoretically as follows:
– Load PCR reaction mixture on a 0.8 – 2% agarose gel.
– Use 1x TAE or 1x TBE as running buffer.
– Electrophorese until DNA band of interest is isolated from adjacent contaminating fragments

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2 Identify bands by staining gel with ethidium bromide.

⚠ ***Wear gloves, ethidium bromide is a known potent carcinogen***

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3 Cut desired DNA band from gel using an ethanol-cleaned scalpel or razor blade.

⚠ ***Minimize gel volume by visualizing DNA and cutting the smallest possible gel slice on a UV light box.***

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4 – Place excised agarose gel slice in a sterile 1.5 ml microcentrifuge tube.
– Determine gel mass by first pre-weighting the tube, and then re-weighting the tube with the excised gel slice.

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5 Add 300 µl Binding Buffer for every 100 mg agarose gel slice to the microcentrifuge tube.

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6 Dissolve agarose gel slice in order to release the DNA:
– Vortex the microcentrifuge tube 15 – 30 s to resuspend the gel slice in the Binding Buffer.
– Incubate the suspension for 10 min at 56°C.
– Vortex the tube briefly every 2 – 3 min during incubation.

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7 After the agarose gel slice is completely dissolved:
– Add 150 µl isopropanol for every 100 mg agarose gel slice to the tube.
– Vortex thoroughly.

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8 Insert one High Pure Filter Tube into one Collection Tube.
– Pipette the entire contents of the microcentrifuge tube into the upper reservoir of the Filter Tube.

⚠ ***Do not exceed 700 µl total volume. If mixture is > 700 µl, split the volume and use two separate Filter Tubes for each portion***

9 Centrifuge 30 – 60 s at maximum speed in a standard table top centrifuge at +15 to +25°C.

10 – Discard the flowthrough solution.
– Reconnect Filter Tube with the same Collection Tube

11 – Add 500 µl Wash Buffer to the upper reservoir.
– Centrifuge 1 min at maximum speed (as above).

12 – Discard the flowthrough solution.
– Recombine Filter Tube with the same Collection Tube.
– Add 200 µl Wash Buffer.
– Centrifuge 1 min at maximum speed.

*i This second 200 µl wash step ensures optimal purity and full removal of Wash Buffer from the glass fibers.*

13 – Discard the flowthrough solution and Collection Tube.
– Recombine Filter Tube with a clean 1.5 ml microcentrifuge tube.

14 – Add 50 – 100 µl Elution Buffer to the upper reservoir of the Filter Tube.
– Centrifuge 1 min at maximum speed.

15 The microcentrifuge tube now contains the purified DNA.

⚠ ***When subsequent $OD_{260}$ determination is planned, centrifuge the eluate for more than 1 min at maximum speed to remove residual glass fibers from the eluate, because they may disturb absorbance measurement. Use an aliquot of the supernatant to determine concentration.***

*i Either use the eluted DNA directly or store the eluted DNA at +2 to +8°C or −15 to −25°C for later analysis.*

# 3. Results

## Purity

Purified DNA is free of small DNA fragments (<100 bp) and other contaminants including mineral oil, primers, salts, unincorporated nucleotides and proteins (*e.g.,* thermostable enzymes) according to the current Quality Control Procedures.

## Expected Yield

Recovery of purified DNA depends on the DNA amount loaded, elution volume, and fragment size. The table below shows expected yields for various DNA amounts, elution volumes and DNA fragment sizes. DNA used was DNA Molecular Weight Marker II.

| DNA MWM II* applied [µg] | Recovery [%] | Elution volume [µl][(1)] | Recovery [%] | Fragment length[(1)(2)] | Recovery [%] |
|---|---|---|---|---|---|
| 5 | 77 | 50 | 68 | < 100 | < 5 |
| 10 | 79 | 100 | 79 | 375 | >95 |
| 25 | 80 | 150 | 80 | 700 | >95 |
| 50 | 56 | 200 | 80 | 3.000 | >95 |

(1) 10 µg DNA were used in this experiment.
(2) Elution volume was 200 µl in this experiment.

# 4. Troubleshooting

| Observation | Possible cause | Recommendation |
|---|---|---|
| Low nucleic acid yield or purity | Kit stored under non-optimal conditions. | Store kit at +15 to +25°C at all times upon arrival. |
| | Buffers or other reagents were exposed to condi-tions that reduced their effectiveness. | Store all buffers at +15 to +25°C. |
| | | Close all reagent bottles tightly after each use to preserve pH, stability, and freedom from contamination. |
| | Ethanol not added to Wash Buffer. | Add absolute ethanol to all Wash Buffers before using. |
| | | After adding ethanol, mix the Wash Buffer well and store at +15 to +25°C. |
| | | Always mark Wash Buffer vial to indicate whether ethanol has been added or not. |
| | Reagents and PCR samples not completely mixed. | Always mix the sample tube well after addition of each reagent. |
| Low recovery of nucleic acids after elution | Non-optimal reagent has been used for elution. Alkaline pH is required for optimal elution. | Do not use water to elute nucleic acids from Filter Tube. |
| | | Use the Elution Buffer in the kit. |
| Subsequent cleavage of the purified PCR DNA product by restriction enzymes is inhibited. | Glass fibers which can coelute with the nucleic acid may inhibit enzyme reactions. | ① After elution step is finished, remove High Pure Filter from tube containing eluted sample and spin this sample tube for 1 minute at maximum speed.<br>② Transfer supernatant into a new tube without disturbing the glass fibers at the bottom of the original tube. |
| Absorbency ($A_{260}$) reading of product too high | Glass fibers which can coelute with nucleic acid, scatter light. | See suggestions under “Subsequent cleavage of the purified PCR product by restriction enzymes is inhibited” above. |
| Purified DNA sample cannot easily be loaded into the well of an agarose gel, but instead “pops out” of the well as it is loaded. | Eluate containing the purified DNA product is contaminated with ethanol from the Wash Buffer. | ① After the last wash step, make certain flowthrough solution containing Wash Buffer does not contact the bottom of the High Pure Filter Tube.<br>② If this has occurred, empty the Collection Tube and reinsert the contaminated filter, and recentrifuge for 30 seconds. |
| Purified DNA is not recovered at high yield. | Reagents exposed to conditions that reduce their effectiveness. | Store all buffers at +15 to +25°C. |
| | | Always mix contents of sample tube thor-oughly after adding each reagent |
| | Not enough Binding Buffer was used for the volume of the DNA sample. | Make sure ratio of PCR sample volume to Binding Buffer is 1:5.<br>⚠ ***Oil overlay, wax, and gel loading dye do not interfere with the purification procedure*** |
| | Incomplete elution. | Elute DNA with two volumes of Elution Buffer (50 µl each): Be sure to centrifuge after each addition of Elution Buffer. |
| Concentration of DNA in the eluate is too low | Low amounts amplified DNA were added to the High Pure Filter Tube (in Step 1) | Decrease the volume of Elution Buffer used to recover DNA.<br>⚠ ***Do not use less than 50 µl Elution Buffer.*** |
| No PCR product in final eluate | No PCR product in starting material. | Check PCR result by agarose gel electro-phoresis before starting purification procedure. |

# 5. Additional Information on this Product

## 5.1. Test Principle

In the presence of the chaotropic salt guanidine thiocyanate, DNA amplified by PCR binds selectively to special glass fibers pre-packed in the High Pure Filter Tube. Bound DNA is purified in a series of rapid wash-and-spin steps to remove contaminating primers, nucleotides, and salts, and then eluted using a low salt solution. This simple method eliminates the need for organic solvent extractions and DNA precipitation, allowing for rapid purification of many samples simultaneously.

① Sample (*e.g.*, PCR reaction) is mixed with Binding Buffer.

② PCR reaction product is bound to the glass fibers pre-packed in the High Pure Filter Tube.

③ Bound DNA is washed to remove unincorporated nucleotides, primers, mineral oil, salts, and the thermostable polymerase.

④ Purified DNA is recovered using the Elution Buffer

### Product Characteristics

Specificity: The special glass fiber fleece only binds DNA fragments with a minimum length of 100 bp, thus oligonucleotides and dimerized primers from PCR reactions are selectively removed.
Recovery: The amount of DNA recovered is dependent on the amount of DNA applied to the glass fiber fleece, the elution volume, and the length of the PCR products. When 5 – 25 µg DNA is applied to the High Pure Filter Tube, approximately 80% of the DNA can be recovered.

## 5.2. References


- Chang PC, Hsieh ML, Shien JH, Graham DA, Lee MS, Shieh HK - Complete nucleotide sequence of avian paramyxovirus type 6 isolated from ducks (2001) *Journal of General Virology* 9, 2157-2168
- D'Errico I, Reyes A, Dinardo MM, Gadaleta G - Study of the mitochondrial transcription factor A (Tfam) gene in the primate Presbytis cristata (2005) *Gene* , 117-124
- Falchetti A, Di Stefano M, Marini F, Del Monte F, Gozzini A, Masi L, Tanini A, Amedei A, Carossino A, Isaia G, Brandi M - Segregation of a M404V mutation of the p62/sequestosome 1 (p62/SQSTM1) gene with polyostotic Paget's disease of bone in an Italian family (2005) *Arthritis Research & Therapy* 6, 1289-1295
- Footitt S, Ingouff M, Clapham D, von Arnold S - Expression of the viviparous 1 (Pavp1) and p34cdc2 protein kinase (cdc2Pa) genes during somatic embryogenesis in Norway spruce (Picea abies [L.] Karst) (2003) *Journal of Experimental Botany* 388, 1711-1719
- Löbner K, Steinbrenner H, Roberts GA, Ling Z, Huang G, Piquer S, Pipeleers DG, Seissler J, Christie MR - Different regulated expression of the tyrosine phosphatase-like proteins IA-2 and phogrin by glucose and insulin in pancreatic islets: Relationship to development of insulin secretory responses in early life (2002) *Diabetes* 10, 2982-2988
- Salesse S, Verfaillie CM - BCR/ABL-mediated Increased Expression of Multiple Known and Novel Genes That May Contribute to the Pathogenesis of Chronic Myelogenous Leukemia (2003) *Molecular Cancer Therapeutics* 2, 173-182


## 5.3. Quality Control

More than 70% recovery is obtained when 10 µg DNA Molecular Weight Marker VIII* mixed with 16 µg bovine serum albumin are applied to the High Pure Filter Tubes. Gel electrophoresis of the DNA eluate confirms the full removal of protein and DNA fragments smaller than 100 bp.

# 6. Supplementary Information

## 6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

| Text convention and symbols | |
|---|---|
| *Information Note: Additional information about the current topic or procedure.* | |
| **Important Note: Information critical to the success of the current procedure or use of the product.** | |
| ① ② ③ etc. | Stages in a process that usually occur in the order listed. |
| ❶ ❷ ❸ etc. | Steps in a procedure that must be performed in the order listed. |
| * (Asterisk) | The Asterisk denotes a product available from Roche Diagnostics. |

## 6.2. Changes to previous version

Correction typo 100 ml to 100 µl.
Editorial changes.

## 6.3. Ordering Information

| Product | Pack Size | Cat. No. |
|---|---|---|
| Reagents , kits | | |
| DNA Molecular Weight Marker VIII | 50 µg in 200 µl, 1 A260 unit 250 µg/ml | 11 336 045 001 |
| DNA Molecular Weight Marker II | 50 µg in 200 µl, 1 A260 unit 250 µg/ml for 50 gel lanes | 10 236 250 001 |
| Agarose LE | 100 g | 11 685 660 001 |
| | 500 g | 11 685 678 001 |
| Agarose MS | 100 g | 11 816 586 001 |
| | 500 g | 11 816 594 001 |
| Agarose MP | 500 g bulk | 03 573 788 001 |
| | 100 g | 11 388 983 001 |
| | 500 g | 11 388 991 001 |

## 6.4. Trademarks

HIGH PURE is trademark of Roche.
All third party product names and trademarks are the property of their respective owners.

## 6.5. License Disclaimer

For patent license limitations for individual products please refer to: **List of LifeScience products**

## 6.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

## 6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

## 6.8. Contact and Support

To ask questions, solve problems, suggest enhancements and report new applications, please visit our **Online Technical Support** Site.

To call, write, fax, or email us, visit **sigma-aldrich.com** and select your home country. Country-specific contact information will be displayed.

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