geneMAG-RNA/DNA 96

compatible for KingFisher[™] 96 and BioSprint[™] 96

Magnetic RNA/DNA purification kit compatible for KingFisher 96[™] or BioSprint 96[™] workstation.



The **geneMAG-RNA/DNA 96** kit is a novel, simple and highly efficient tool for the isolation of total RNA/DNA with magnetic silica beads.

Simple washing steps with two different buffers remove salts, metabolites and macromolecular cellular components. Pure RNA/DNA is finally eluted under low ionic strength conditions with RNase-free water.



The magnetic bead processing of KingFisher™ 96 / BioSprint™ 96 workstation

The KingFisher™ 96 workstation is a trademark of Thermo Fisher Scientific.

The BioSprint[™] 96 workstation is a trademark of Qiagen.

Kits	Contents	Number of isolations	Price Euro/US\$
geneMAG-RNA/DNA 96 (Cat. No.: KF3401-96)	 100 ml Lysis & Binding Buffer 200 ml Wash Buffer I 10 ml SiMAG/KF-DNA Beads 	1 x 96 preps per 10º bacteria	185 / 240
geneMAG-RNA/DNA 480 (Cat. No.: KF3401- 480)	 500 ml Lysis & Binding Buffer 1000 ml Wash Buffer I 50 ml SiMAG/KF-DNA Beads 	5 x 96 preps per 10º bacteria	725 / 942

Reagents and Equipment to be Supplied by the User

- Wash Buffer II: 70% Ethanol or 70% Isopropanol.
- Elution Buffer: Nuclease-free water or DEPC-Water for elution of RNA/DNA from the beads.
- KingFisher[™] 96 / BioSprint[™] 96 workstation
- Deep well 96-well plates (2,2 ml) squared well
- KingFisher[™] 96 plate (0,3 ml)
- Magnet Head for deep well 96-well plates
- DNase Treatment: DNA-free™ Kit, DNase Treatment and Removal Reagents Part Number AM1906 (Applied Biosystems).

Safety Note

Lysis/Binding-Buffer and **Wash Buffer I** contain chaotropic salts, which are irritant. Take appropriate laboratory safety measures and wear gloves when handling. <u>Avoid skin and eye contact</u>

Protocol for KingFisher[™] 96 or BioSprint 96[™]

This protocol describes the isolation of genomic-DNA from 10⁹ bacteria cells per each well of 96-well plate with the geneMAG-RNA/DNA 96 kit using KingFisher™ 96 or BioSprint™ 96 workstation.

Preparation of the deep well 96-well plates (2,2 ml)

First 96-well plate:

1. Add 100 µl Bacteria Suspension in each well

Bacteria Suspension: Add 1.5 ml of overnight cultured cells (approximately 10⁸ cells) into a 1.5 ml microcentrifuge tube. Centrifuge for 2 minutes at 11,000 x g to pellet the cells. Discard the supernatant. Resuspend the bacteria pellet in 100 μl Lysis & Binding Buffer

2. Add 1000 µl Lysis & Binding Buffer and 100 µl SiMAG/KF-DNA.

Second 96-well plate:

1. Add 1000 µl Wash Buffer I in each well

Third 96-well plate:

1. Add 1000 µl Wash Buffer I in each well

Fourth 96-well plate:

1. Add 1000 µl Wash Buffer II (70% 2-propanol) in each well

Fifth 96-well plate:

1. Add 1000 µl Wash Buffer II (70% 2-propanol) in each well

Sixth 96-well plate: Use 96-well plate with max. volume of 0,3 ml

1. Add 100 µl Elution Buffer (dH2O) in each well

Seventh 96-well plate: Parking station!!!!

Protocol for KingFisher[™] 96 or BioSprint 96[™]

Settings of the processing times for Lysis- & Binding-, Wash- and Elution steps :

Start the KingFisher Software and set the following parameters:

- 1. Lysis & Binding process (first deep-well plate): 6 minutes with low stirring
- 2. Wash process with Wash Buffer I (second deep-well plate): 1 minutes with medium stirring
- **3.** Wash process with Wash Buffer I (third deep-well plate): 1 minutes with medium stirring
- 4. Wash process with Wash Buffer II (fourth deep-well plate): 1 minutes with medium stirring
- **5.** Wash process with Wash Buffer II (fifth deep-well plate): 1 minutes with medium stirring
- 6. Elution process with Elution Buffer (dH2O): Heat time: 10 minutes with high stirring Temperature: 80°C



Total RNA/DNA was isolated from 1.5ml E. coli LB culture using geneMAG-RNA/DNA 96. (Data kindly provided by Cengiz Öztürk, Charité, University Hospital of Humboldt-University to Berlin, Germany)

Protocol

Optional: DNase Treatment

Use Ambion® DNA-free[™] DNase Treatment and Removal Reagents and follow the instructions of the manufacturer.

- **10.** Add 5 μ L (2 Units/ μ I) rDNase I to the eluted RNA/DNA and mix gently.
- **11.** Incubate at 37°C for 30 min.
- 12. Add 10 µl resuspended DNase Inactivation Reagent and mix well.
- 13. Incubate 2 minutes at room temperature, mix occasionally.
- **14.** Centrifuge at 11,000 x g for 2 minutes, carefully transfer the RNA containing supernatant into a fresh tube.



Total RNA was isolated from 1 ml E. coli LB culture using geneMAG-RNA/DNA 96 with subsequent DNase treatment (Ambion).

(Data kindly provided by Cengiz Öztürk, Charité, University Hospital of Humboldt-University to Berlin, Germany)

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