EXTRACTME®
DNA YEAST KIT

Cat. No. EM10
Version: 1.2018

Kit for genomic DNA isolation from yeast

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I. INTENDED USE

The ***EXTRACTME*** **DNA YEAST KIT** is designed for a rapid and efficient purification of high quality DNA from broth and plate yeast cultures as well as frozen cells. The isolation protocol and buffer formulations were optimized for high isolation efficiency and DNA purity. The product is intended for research use only.

II. COMPONENTS OF THE KIT AND STORAGE CONDITIONS

NUMBER OF ISOLATIONS	10 ISOLATIONS	50 ISOLATIONS	250 ISOLATIONS	Storage Conditions[1]
Catalogue number	EM10-010	EM10-050	EM10-250	
YS Buffer (Spheroplast Buffer)	2 ml	10 ml	50 ml	RT
⚠ **Lysis Mix**	20 µl	100 µl	500 µl	-20°C
YL Buffer (Lysis Buffer)	3 ml	15 ml	75 ml	RT
⚠ **RNase A**[*] (lyophilized)	1 tube	1 tube	5 tubes	-20°C[2]
RNase Buffer	100 µl	220 µl	1.1 ml	RT
⚠ **Proteinase K**[**] (lyophilized)	1 tube	1 tube	5 tubes	-20°C[3]
Proteinase Buffer	200 µl	560 µl	2.8 ml	RT
YB Buffer (Binding Buffer)	3.5 ml	18 ml	88 ml	RT
YW Buffer (Wash Buffer)	10 ml	50 ml	250 ml	RT
Elution Buffer	2 ml	10 ml	5x 10 ml	RT
DNA Purification Columns	10 pcs	50 pcs	5x 50 pcs	RT
Collection Tubes (2 ml)	10 pcs	50 pcs	5x 50 pcs	RT

[1] RT – room temperature (+15°C to +25°C)

[2] After reconstitution, **RNase A** should be kept **at +4°C for short term storage (several days) or in aliquots at -20°C**.

[3] After reconstitution, **Proteinase K** should be kept **at -20°C**.

[*] Prior to the first use, add 220 µl RNase Buffer to a tube containing RNase A lyophilizate (in the kit for 10 isolations 100 µl of a buffer should be added).

[**] Prior to the first use, add 560 µl Proteinase Buffer to a tube containing Proteinase K lyopilizate (in the kit for 10 isolations 200 µl of a buffer should be added).

In order to avoid evaporation, ensure that the buffer bottles are tightly closed before storing.

Expiry date
Under proper storage conditions the kit will remain stable for at least 12 months from opening or until the expiry date.

III. ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- 100% β-mercaptoethanol or 1M dithiothreitol (DTT)
- 1.5–2 ml sterile microcentrifuge tubes
- automatic pipettes and pipette tips
- disposable gloves
- microcentrifuge with rotor for 1.5–2 ml (≥ 11 000 x g)
- dry block heater or water bath (up to 55°C)
- vortex mixer

IV. PRINCIPLE

DNA purification procedure consists of five steps and utilizes spin minicolumns with membranes which efficiently and selectively bind nucleic acids. During the first step cell walls of the yeast cells are disintegrated in the YS Buffer (Spheroplast Buffer). Spheroplasts obtained this way are enzymatically lysed by Proteinase K in lysis buffer. To obtain an RNA-free DNA sample, RNA is removed by RNase A. After the addition of chaotropic salts, the lysate is applied to the purification column membrane and DNA is bound. The two-step washing stage effectively removes impurities and enzyme inhibitors. Purified DNA is eluted with then use of a low ionic strength buffer (Elution Buffer) or water (pH 7.0–9.0) and may be used directly in all downstream applications such as PCR, qPCR , Southern blotting, DNA sequencing, enzymatic restriction, ligation and so forth, or stored until ready to use.

V. QUALITY CONTROL

The quality of each production batch (LOT) of the ***EXTRACTME*** **DNA YEAST KIT** is tested with the use of standard QC procedures. Purified DNA concentration and quality are evaluated by gel electrophoresis and spectrophotometer.

VI. PRODUCT SPECIFICATIONS

SAMPLE MATERIAL
Broth or plate yeast culture, frozen cell pellet
EFFICIENCY
Up to 1 x 10^8 cells → 100% 2 x 10^8 ÷ 9 x 10^8 → 30–70%
BINDING CAPACITY
Approx. 40 µg DNA
TIME REQUIRED
Approx. 75 minutes (including incubation time)
DNA PURITY
A_{260}/A_{280} ratio = 1.7 – 1.9

VII. SAFETY PRECAUTIONS

- Yeast culture is treated as a biohazardous material of its potential pathogen content or health and life-threatening substances. While working with yeast cultures it is essential to comply with all safety requirements for working with biohazardous material.
- It is advisable to conduct the entire isolation procedure in a Class II Biological Safety Cabinet or at laboratory burner as well as wearing disposable gloves and suitable lab coat at all times.
- Use of sterile pipette filter tips is recommended.
- Avoid the cross-contamination of DNA between minicolumns.
- Guanidine salts' residues may form highly reactive components when combined with oxidation compounds. In case of spillage, clean the surface with a detergent-water solution.
- In case of spillage of a liquid containing microorganisms, clean the contaminated surface with a detergent-water solution.

VIII. RECOMMENDATIONS AND IMPORTANT NOTES

Sample material

DNA isolation efficiency and purity can depend not only on the number of yeast cells, cell type (morphology) or antibiotics in the grow medium, but also on the age and condition of the yeast cells.

The kit is designed to isolate DNA from max. 1×10^8 yeast cells. Using greater number of cells may significantly reduce the isolation efficiency and the purity of the DNA.

Extracting DNA from fresh starting material or frozen cell pellets is recommended. To minimize DNA degradation, avoid subjecting the sample material to repeated freeze/thaw cycles. Using old or repeatedly frozen/thawed material may result in low efficiency isolation of high molecular DNA.

Addition of reducing agents

In order to aid lysis of the yeast cell wall, a β-mercaptoethanol (β-ME) or dithiothreitol (DTT) should be added to YS Buffer. These compounds are the reducing agents of the disulphide bridges and additional disintegrating factors of the yeast cell wall, as well as is Lysis Mix. The use of additional reducing agents is optional.

Preparation of YS Buffer

When isolating DNA from a number of samples, it is more convenient to prepare YS Buffer with β-ME or DTT before commencing the isolation. For the buffer preparation, the quantity of YS Buffer sufficient for the desired number of isolations should be transferred to a sterile 1.5 ml or falcon tube. For every 1 ml of YS Buffer, 1 µl of 14M β-ME or 50 µl of 1M DTT should be added and mixed thoroughly. Suspend the yeast cell pellets in **200 µl** of **YS Buffer** prepared in this way and add **2 µl** of **Lysis Mix**, **4 µl** of **RNase A**. Mix by vortexing. Continue the isolation from step 4 of the Isolation Protocol (section XI).

DNA elution

An optimal volume of Elution Buffer used should be chosen in accordance with the quantity of sample material and final DNA concentration expected. The use of 50-100 µl Elution Buffer is recommended when extracting from no more than 10^8 cells, 200 µl while extracting from greater number of cells.

If a high DNA concentration is desired, the elution's volume may be reduced to 20 µl. However, it may reduce the efficiency. It is essential to apply Elution Buffer precisely to the centre of the membrane.

In order to maximize the DNA retrieval, heat Elution Buffer to 80°C and incubate it on the membrane for 10 minutes.

If full DNA retrieval is required, a second elution should be performed. For the second elution, repeat steps 22–25 of the Isolation Protocol (see section XI), placing DNA Purification Column in a new, sterile 1.5 ml Eppendorf tube.

Elution Buffer does not contain EDTA, which may interfere with some enzymatic reactions. If desired, DNA can alternatively be eluted from the membrane with nuclease-free water, pH 7.0-9.0.

IX. SAMPLE PREPARATION

Isolation from broth culture (0.2–3 ml)

Before starting, always mix broth culture thoroughly. Transfer a desired quantity of yeast culture (no more than 1.5 ml) to a sterile 1.5 ml Eppendorf tube and spin at 3000–4000 x g. Remove the supernatant. If the isolation from more than 1.5 ml of culture is required, add another 1.5 ml of the culture to the top of the cell pellet thus obtained and spin again. Continue the isolation following the Isolation Protocol from step 2 (section XI).

Isolation from frozen cells

Immediately after retrieving the frozen cell pellet from the freezer, resuspend it in **200 µl of YS Buffer**. Do not let the cell pellet to thaw. Continue the isolation following the Isolation Protocol from step 3 (section XI).

Isolation from plate culture

Transfer **200 µl of YS Buffer** to a sterile 1.5 ml Eppendorf tube. Using a loop, take a sufficient amount of cell culture from the plate and resuspend it in YS Buffer. Continue the isolation following the Isolation Protocol from step 3 (section XI).

X. PRIOR TO ISOLATION

1. Mix well each buffer supplied with the kit.
2. Prepare **Proteinase K** solution by reconstituting the lyophilizate in an appropriate quantity of Proteinase Buffer. Reconstitute **RNase A** lyophilizate in an appropriate amount of RNase Buffer.
3. Examine the buffers. If a sediment has occurred in any of them, incubate it at **37°C** (**YW Buffer**) or at **50–60°C** (other buffers), mixing occasionally until the sediment has dissolved. Cool to room temperature.
4. Set a dry block heater or water bath to **30°C** and **55°C**.
5. Unless otherwise stated, conduct all the isolation steps at room temperature.

XI. ISOLATION PROTOCOL

1. Pellet cells from 0.2–1.5 ml yeast broth culture by centrifugation for 5 min at 3000–4000 x g.
   - ⚠ Other culture volumes in the 0.2–3 ml range may be used. For instructions, see section IX. Sample preparation.
2. Discard the supernatant, and resuspend the cell pellet thoroughly in **200 µl YS Buffer**.
3. Add **2 µl Lysis Mix**, **4 µl RNase A** and **0.2 µl 100% β-ME** (optional, not included in the kit)
   - ⚠ It is more convenient to prepare YS Buffer with β-ME before commencing the isolation, when isolating DNA from a number of samples. For instructions, see section VIII. Recommendations and Important Notes.
   - ⚠ 10 µl of 1M DTT can be added instead of β-ME. Alternatively, the addition of a reducing agent can be skipped, however the Lysis Mix will not reach its full activity.
4. Incubate at **30°C** for 30 min, mixing occasionally by inverting.
5. After incubation, centrifuge for 2–8 min at 1000 x g.
6. Carefully, remove the supernatant with a pipette tip. DO NOT disturb the spheroplasts pellet!
7. Suspend the cell pellet thoroughly in **300 µl YL Buffer**.
8. Add **10 µl Proteinase K** and mix by vortexing.
9. Incubate at **55°C** for 10 min.
10. Add **350 µl YB** Buffer and mix thoroughly.
11. Incubate for an additional 5 min at **55°C**.
12. After incubation vortex vigorously for 15 s.
13. Centrifuge for 120 s at 11 000 – 15 000 x g.

14. Carefully pipet the **supernatant** containing DNA into a DNA Purification Column placed in a Collection Tube. Centrifuge for 60 s at 11 000 – 15 000 x g.
    ⚠ Keep the pipette tip away from the pellet.
15. Transfer the DNA Purification Column to a new Collection Tube.
16. Add **600 µl YW Buffer** and centrifuge for 30 s at 11 000 – 15 000 x g.
17. Discard a filtrate and reuse the Collection Tube.
18. Add **400 µl YW Buffer** and centrifuge for 30 s at 11 000 – 15 000 x g.
19. Discard the filtrate and reuse the Collection Tube.
20. Centrifuge for 60–120 s at 15 000 – 21 000 x g.
    ⚠ Wash buffer contains alcohol, which may interfere with some enzymatic reactions and also decrease the elution efficiency. It is therefore vital to remove alcohol completely from the DNA Purification Column prior to elution.
21. Discard the Collection Tube and the filtrate and carefully transfer the DNA Purification Column to a sterile 1.5 ml Eppendorf microcentrifuge tube.
22. Add **50–100 µl Elution Buffer**, directly onto the DNA Purification Column membrane.
    ⚠ Other buffer volumes between 20–100 µl range may be used. For instructions, see section VIII. Recommendations and important notes.
23. Incubate the DNA Purification Column at room temperature for 120 s.
24. Centrifuge at 11 000 – 15 000 x g for 60 s.
25. Remove the DNA Purification Column. Isolated DNA should be stored at **+4°C** or **-20°C** depending on further applications.

XII. TROUBLESHOOTING

Problem	Possible cause	Solution
Incomplete cell wall lysis.	Too many cells were taken for DNA isolation.	Reduce an amount of starting material.
	No reducing agent added.	Repeat the isolation, ensuring that an appropriate quantity of β-ME or DTT has been added.
	Reduced efficiency of Lysis Mix.	Use fresh Lysis Mix solution. Ensure that Lysis Mix is stored at -20°C.
	Insufficient lysis time for a material taken for DNA isolation.	Extend the incubation time at 30°C to at least 1 h.
Column becomes clogged during purification.	Incomplete cell lysis.	See „Incomplete cell wall lysis".
	A thick slime of DNA was formed.	Repeat centrifugation for 60 s at 21 000 x g or at maximum speed.
Low yield of purified DNA.	Few yeast cells in starting material.	Increase an amount of starting material or decrease a volume of Elution Buffer to 20 µl.
	Incomplete cell lysis.	See „Incomplete cell lysis".
	Reduced Proteinase K activity.	Prepare a fresh Proteinase K solution. Ensure that Proteinase K solution is stored at -20°C.
	The lysate was not mixed sufficiently with the binding buffer YB.	Repeat the isolation, paying a particular attention to whether the lysate is thoroughly mixed with the YB Buffer (Step 10) before incubating at 55°C.
	Purified DNA contains residual alcohol.	Repeat the isolation, paying a particular attention to whether no residual YW Buffer is left in the DNA Purification Column after centrifugation in step 20.
	Incomplete DNA elution from the membrane.	Before applying Elution Buffer to the membrane, heat it to 80°C. Apply Elution Buffer directly to the centre of the membrane. Extend the incubation time with Elution Buffer to 10 min. Perform second elution. Increase volume of Elution Buffer to 200 µl.
	The pH of the water used for elution is lower than 7.0.	Use Elution Buffer for DNA elution.

Problem	Possible cause	Solution
Low concentration of purified DNA.	Too much Elution Buffer was used.	Decrease the volume of Elution Buffer. For details, see section VIII. Recommendations and important notes.
Isolated DNA is of low purity.	Incomplete protein degradation.	Prepare a fresh Proteinase K solution. Ensure that the Proteinase K solution is stored at -20°C.
	One of the washing steps was omitted.	Repeat the isolation, performing both washing steps.
	Purified DNA contains residual alcohol.	Repeat the isolation, paying a particular attention to whether no residual YW Buffer is left in the DNA Purifiaction Column after centrifugation in step 20.
DNA flows out of the lanes in the agarose gel.	Purified DNA contains residual alcohol.	Repeat the isolation, paying a particular attention to whether no residual YW Buffer is left in the DNA Purifiaction Column after centrifugation in step 20.
Purified DNA is degraded.	Old or damaged material was used.	It is recommended to perform an isolation from fresh overnight yeast broth culture. Older yeast cultures may contain degraded DNA.
	Inappropriate sample storage conditions.	Always perform an isolation from fresh or frozen material. Avoid subjecting a sample material to repeated freeze/thaw cycles.
RNA contamination present.	Reduced RNase A activity.	Prepare a fresh RNase A solution and repeat the isolation. Ensure proper storage conditions.
	Insufficient incubation time with RNase A.	Extend an incubation time with the RNase A (step 4) to 1 hour.
Inhibition of downstream enzymatic reactions.	Purified DNA contains residual alcohol.	Repeat the isolation, paying a particular attention to whether no residual YW Buffer is left in the DNA Purifiaction Column after centrifugation process during step 20.
	Insufficient DNA in a sample.	See „Incomplete cell lysis“ and „Low concentration of purified DNA“.
	High RNA contamination.	See „RNA contamination present“.

XIII. SAFETY INFORMATION

Proteinase K

Danger
H315, H319, H334, H335
P261, P271, P304+P340, P342+P311, EUH208

YB Buffer

Danger
H315, H318, H412
P264, P280, P305+P351+P338 P310

YW Buffer

Danger
H225, H319, H336
P210, P261, P305+P351+P338, P304+P340 P312

EUH208 Contains Proteinase. May produce an allergic reaction. **H225** Highly flammable liquid and vapour. **H315** Causes skin irritation. **H318** Causes serious eye damage. **H319** Causes serious eye irritation. **H334** May cause allergy or asthma symptoms or breathing difficulties if inhaled. **H335** May cause respiratory irritation. **H336** May cause drowsiness or dizziness. **H412** Harmful to aquatic life with long-lasting effects. **P210** Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. **P261** Avoid breathing dust/fumes/gas/mist/vapours/spray. **P264** Wash hands thoroughly after handling. **P271** Use only outdoors or in a well-ventilated area. **P280** Wear protective gloves/protective clothing/eye protection/face protection. **P305+P351+P338** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **P342+P311** If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician. **P304+P340** IF INHALED: remove person to fresh air and keep comfortable for breathing. **P312** Call a POISON CENTER/doctor if you feel unwell. **P310** Immediately call a POISON CENTER/doctor.