ab206997
Yeast Nuclei Isolation Kit

Instructions for use:

For fast and easy purification of nuclei from yeast cells.

This product is for research use only and is not intended for diagnostic use.

Version 5 Last Updated 01 February 2019

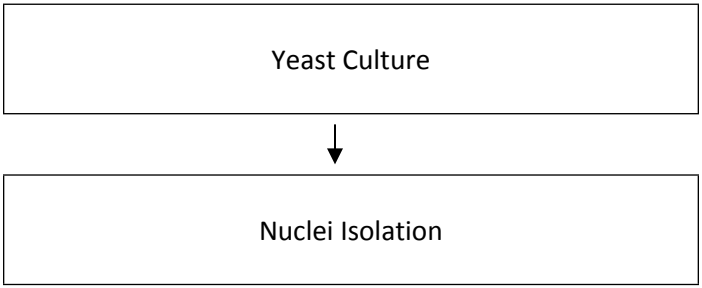
Table of Contents

1. BACKGROUND

Abcam's Yeast nuclei isolation kit (ab206997) provides an easy and fast method for isolation of nuclei from yeast cells for downstream applications such as DNA-protein interaction, RNA-protein interaction and protein-protein interaction studies, DNase I foot-printing analysis, enzymatic assays, pull-down assays, as well as western blot and ELISA.

To understand the nature of the replication and expression of the yeast genome, it is essential to have a method for the preparation of nuclei at different stages of growth. Abcam's Yeast nuclei isolation kit enables easy and fast purification of nuclei from yeast cells using yeast cell wall lysis and homogenization.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 6 and 9.

5. LIMITATIONS

- Kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Buffer A	50 mL	-20°C	4°C
Buffer B	50 mL	-20°C	4°C
1 M DTT	1 mL	-20°C	-20°C
Lysis Enzyme Cocktail	500 µL	-20°C	-20°C
Buffer C	90 mL	-20°C	4°C
Protease Inhibitor Cocktail (Lyophilized) (22mg)	1 vial	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- Media to grow yeast cells
- Dounce tissue homogenizer
- Microscope to visualize nucleus
- Refrigerated centrifuge
- DMSO

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single isolation experiment. The starting amount of tissue or cells for a single experiment will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Storage conditions: For intact nuclei, snap freeze in liquid nitrogen and store frozen nuclei at -80°C. For gel loading purposes, nuclei can be stored in Lysis Buffer or SDS PAGE loading dye (not provided).

9. REAGENT PREPARATION

9.1. **Buffer A:**

Store at 4°C. Warm the required volume of Buffer A to room temperature and add DTT to a final concentration of 10 mM prior to use.

9.2. **Buffer B:**

Store at 4°C. Warm the required volume of Buffer B to room temperature.

9.3. **1 M DTT:**

Ready to use as supplied. Aliquot and store at -20°C.

9.4. **Lysis Enzyme Cocktail:**

Ready to use as supplied. Aliquot and store at -20°C.

9.5. **Buffer C:**

Store at 4°C. Keep on ice while in use. Add Protease Inhibitor Cocktail (1:1000) to the required volume of Buffer C before use.

9.6. **Protease Inhibitor Cocktail (lyophilized) (22 mg):**

Resuspend Protease Inhibitor Cocktail in 250 µL of DMSO. Store at -20°C.

10. SAMPLE PREPARATION

- The described procedure is for small-scale isolation (10-20 mL) for total OD~20. For a large-scale preparation (total OD~200), calculate the reagent volumes accordingly.

10.1. Grow yeast cells in an appropriate media overnight at 30°C with shaking at 200 rpm in an orbital shaker. For temperature sensitive mutants use the appropriate temperature.

10.2. When cells are in log phase, determine the OD of the culture at 600 nm. Multiply the OD by the total volume of the culture (mL) to calculate the total OD.

11. ASSAY PROCEDURE

11.1. Centrifuge the yeast culture at 3,000 x g for 5 minutes at room temperature and discard the supernatant.

11.2. Wash the cells by resuspending in two volumes of ultrapure water.

11.3. Re-suspend the cell pellet in 1 mL of Buffer A (containing DTT) and incubate at 30°C for 10 minutes with gentle shaking.

11.4. Centrifuge at 1,500 x g for 5 minutes at room temperature and discard the supernatant.

11.5. Resuspend the cell pellet in 1 mL of Buffer B. Aliquot 10 µL suspension into a separate glass tube (control sample).

11.6. Add 10 µL Lysis Enzyme Cocktail to the remaining cell suspension and incubate for 10-15 minutes at 30°C with shaking. Aliquot 10 µL of suspension into a clean glass tube.

Note: To check the efficiency of spheroplast formation, add 990 µL of water to 10 µL aliquot from steps 11.5 and 11.6 (Control and with Lysis Enzyme Cocktail respectively). Measure OD at 600 nm. Incubation should continue until the OD of the sample is decreased 30-40% after adding Lysis Enzyme Cocktail compared to the control.

11.7. Centrifuge spheroplasts at 1,500 x g for 5 minutes and discard the supernatant. From this step onwards, keep the tubes on ice.

11.8. Resuspend the spheroplast pellet in 1 mL of Buffer C with protease inhibitor cocktail.

11.9. Transfer the suspension to a glass Dounce homogenizer and lyse with 3-5 strokes on ice.

11.10. Shake the suspension for 30 minutes at room temperature.

11.11. Centrifuge at 1,500 x g for 5 minutes at 4°C to remove the debris. Collect the supernatant.

11.12. Centrifuge at 20,000 x g for 10 minutes at 4°C to pellet the nuclei. Discard the supernatant and resuspend the nuclei pellet in Buffer C.

11.13. Determine the protein concentration and adjust the desired protein concentration by adding Buffer C.

11.14. Check the quality of nuclei under a light microscope or by adding 4 µL nuclei to 4 µL of DAPI (1µg/mL) and viewing under a fluorescence microscope.

12. TYPICAL DATA

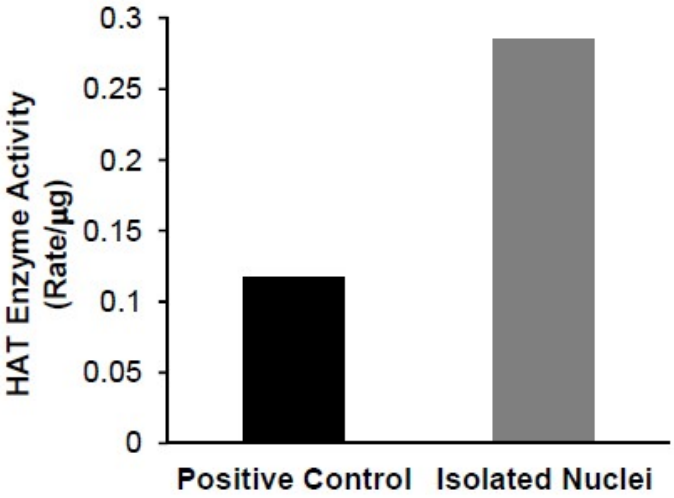


Figure 1: Functional Activity of Isolated Yeast Nuclei. Purified yeast nuclei were analyzed for HAT enzyme activity using H3 substrate. In yeast, H3-dependent HAT activity is nuclear specific. Positive Control: Nuclear Extract (NE, 4 mg/mL). Purified yeast nuclei show significant HAT Enzyme Activity. Yeast nuclei were isolated following the kit protocol.

13. NOTES

RESOURCES

RESOURCES

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940