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Yeast Nuclei Isolation Kit

rev. 09/13

(Catalog # K289-50; 50 assays; Store at -20°C)

I. Introduction:

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. BioVision's Yeast Nuclei Isolation kit enables fast and easy purification of nuclei from yeast cells, utilizing yeast cell wall lysis and homogenization.

II. Application:

- DNA-Protein interaction, RNA-Protein interaction and Protein-Protein interaction studies
- DNase I footprinting analysis, Enzymatic Assays and Pull-down assay
- Western blot and ELISA

III. Sample Type:

Yeast cell culture

IV. Kit Contents:

Components	K289-50	Cap Code	Part Number
Buffer A	50 ml	Blue	K289-50-1
Buffer B	50 ml	NM	K289-50-2
1 M DTT	1 ml	Green	K289-50-3
Lysis Enzyme Mix	500 µl	Orange	K289-50-4
Buffer N	90 ml	Amber	K289-50-5
Protease Inhibitor Cocktail (Lyophilized)	1 Vial	Red	K289-50-6

V. User Supplied Reagents & Equipment:

- Media to grow yeast cells
- Dounce Tissue homogenizer (cat. # 1998)
- Microscope to visualize nucleus
- Refrigerated centrifuge

VI. Storage and Handling:

Store kit at -20°C. Warm Buffers A and B to room temperature (RT) before use. Read the entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

- **Buffer A:** Store at 4°C. Warm to RT & add DTT to final conc. of 10 mM prior to use to the desired volume of buffer.
- **Buffer B:** Store at 4°C. Warm to RT & add Lysis Enzyme Mix to the desired volume of Buffer (5 µl/ml of Buffer) just before use.
- **Lysis Enzyme Mix:** Aliquot and store at -20°C.
- **Buffer N:** Store at 4°C. Keep on ice while in use. Add Protease Inhibitor Cocktail (1:1000) before use or as needed.
- **Protease Inhibitor Cocktail:** Resuspend Protease Inhibitor Cocktail in 250 µl of DMSO. Store at -20°C.

VIII. Yeast Nuclei Isolation Protocol:

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.

1. **Yeast Culture:** Grow yeast cells in appropriate media overnight at 30°C, shaking at 200 rpm. For temperature-sensitive mutants use the appropriate temperature. 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.

2. **Nuclei Isolation:**

2.1 Centrifuge the yeast culture at 3,000 x g at RT for 5 min. and discard the supernatant. Wash the cells by resuspending in 2 volumes of ultrapure water. Resuspend the cell pellet in 1 ml of Buffer A containing DTT and incubate for 10 min. at 30°C with gentle shaking. Centrifuge at 1,500 x g at RT for 5 min. and discard the supernatant.

2.2 Resuspend the cell pellet in 1 ml of Buffer B. Aliquot 10 µl suspension in separate glass tube (Control). Add 10 µl Lysis Enzyme Mix to the remaining cell suspension and incubate for 10-15 min. at 30°C in shaking incubator. Aliquot 10 µl of suspension again in another glass tube.

Note: To check the efficiency of spheroplast formation, add 990 µl of water to 10 µl aliquot from step 2.2 (Control & with Lysis Enzyme Mix). Measure OD at 600 nm. Incubation should continue until the OD of the sample is decreased 30-40% after adding Lysis Enzyme Mix compared to Control.

2.3 Centrifuge spheroplasts at 1,500 X g for 5 min. and discard the supernatant. From this step onwards, keep the tubes on ice. Resuspend the spheroplast pellet in 1 ml of Buffer N with protease inhibitor cocktail. Transfer the suspension to a Glass Dounce homogenizer (not provided) and stroke 3-5 times on ice. Shake the suspension for 30 min. at RT. Centrifuge at 1,500 x g for 5 min. at 4°C to remove the debris. Collect the supernatant. Centrifuge at 20,000 x g for 10 min. at 4°C to pellet the nuclei. Discard the supernatant and resuspend the nuclei pellet in Buffer N. Determine the protein concentration and adjust the desired protein

concentration by Buffer N accordingly. Check the quality of nuclei under light microscope or with DAPI by adding 4 µl nuclei to 4 µl of DAPI (1µg/ml) and view under the fluorescence microscope.

Note: Application Based 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 (cat. # 1067) or SDS PAGE loading dye (Not provided).

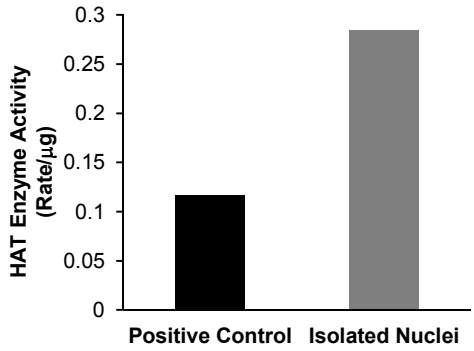

Figure: Functional Activity of Isolated Yeast Nuclei: Purified yeast nuclei were analyzed for HAT enzyme activity using H3 substrate by BioVision's HAT Activity Colorimetric Assay Kit (K332). In yeast, H3-dependent HAT activity is nuclear specific. Positive Control: Nuclear Extract (NE, 4 mg/ml) (K332-100-5). Purified yeast nuclei show significant HAT Enzyme Activity. Yeast nuclei were isolated following the kit protocol.

IX. RELATED PRODUCTS

Yeast Mitochondria Isolation Kit (K259)
Yeast Mitochondria (*Pichia pastoris*) (1111)
Mitochondria/Cytosol Fractionation Kit (K256)
Nuclear/cytosol Fractionation Kit (K266)
FractionPREP™ Cell Fractionation Kit (K270)
EZLys™ Yeast Protein Extraction Reagent (8003)
Yeast Mitochondria (*S. cerevisiae*) (1222)
Cytosol/Particulate Separation Kit (K267-50)
Mammalian Cell & Tissue Extraction Kit (K269)
Dounce Tissue Homogenizer (1998)

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