

Plant Nucleus Resuspension Buffer

Cat. No.: P1509601 | Pack size: 100 mL | Storage: -20 °C

Overview

The nucleus is a functional unit that fully preserves genetic material and directs RNA synthesis. RNA is essential for the synthesis of proteins and other cellular components. In most cell types, the nucleus is anchored within the cell due to the continuity between the nuclear envelope and the endoplasmic reticulum, as well as the connections between the nuclear surface and the cytoskeleton. Flow cytometry is a rapid, accurate, and efficient method for determining and analyzing cellular DNA content. In recent years, it has been widely applied in plant research, such as cell cycle analysis, plant ploidy identification, chromosome sorting, nuclear DNA content measurement, reproductive pathway identification, DNA variation analysis, genetic stability analysis, and somatic embryogenesis analysis. Physical methods alone, such as chopping leaves, often fail to yield a large number of intact nuclei. It is also necessary to add buffer solutions containing specific components to disrupt plant cells, disperse organelles, and further dissociate nuclei, laying the foundation for subsequent nuclear DNA extraction and DNA content analysis. Biological cells to be analyzed by flow cytometry must be in a single-cell suspension state. The key to preparing high-quality nuclear suspensions lies in selecting an appropriate nuclear isolation buffer. There are significant differences in tissue structure and chemical composition among different plant species, leading to varying effectiveness of nuclear isolation buffers. Currently, there is no universally applicable nuclear isolation buffer. Therefore, it is necessary to test different buffers or even modify their components to achieve the optimal nuclear isolation effect.

Nuclear Separation Buffer (NSB), also known as Dissociation Buffer, can separate the nucleus from intracellular components such as the cell membrane, endoplasmic reticulum, and cytoskeleton. Commonly used dissociation buffers at present include Galbraith, Tris-MgCl₂, HEPES, WPB, LB01, mGb, Marie, GPB, OTTO, etc. Each type of dissociation buffer varies in composition and concentration, and different plant samples require the selection of a suitable matching dissociation buffer. Plant Nucleus Resuspension Buffer is mainly composed of 20 mM HEPES, sucrose, magnesium chloride, DTT, glycerol, etc., with a pH of 7.8±0.1. After plant tissue is treated with Nuclear Isolation Buffer, Plant Nucleus Resuspension Buffer is used for the resuspension and preservation of nuclei. This reagent is for research use only and not intended for clinical diagnosis or any other purposes.

Operating Procedures (for reference only)

Micromethod for Nuclear Extraction

1. Take 0.1-0.5 g of fresh plant sample, rinse it with distilled water, blot dry with filter paper, chop it into pieces with a sharp blade, place it in a homogenizer, add 2 ml of self-prepared dissociation buffer, and homogenize for 1-2 minutes.
2. Filter the homogenate through two layers of sterile plain gauze, rinse the homogenizer and gauze with another 2 ml of dissociation buffer, and combine the filtrates.
3. Centrifuge the combined filtrate at 70 rpm for 5 minutes at room temperature. Transfer the supernatant to a new centrifuge tube, then centrifuge at 700 rpm for 15 minutes. Discard the supernatant and retain the nuclear pellet at the bottom.
4. The nuclear pellet can be resuspended in Plant Nucleus Resuspension Buffer and stored at -70°C for downstream experiments such as nuclear lysis, nuclear DNA extraction, and purification.

Precautions

1. Insufficient sample amount or inadequate homogenization may result in a low yield of nuclei, which will affect subsequent experiments.
2. Inappropriate selection of dissociation buffer, improper sample storage, repeated freeze-thaw cycles of samples, or overly vigorous extraction processes may cause fragmentation or degradation of the extracted DNA.
3. Plant Nucleus Resuspension Buffer contains sucrose and HEPES, which are susceptible to bacterial contamination. It is recommended to aliquot the buffer into small portions and store them frozen immediately after the first dissolution.
4. See the attached table for "Components and Functions of Different Dissociation Buffers" and "Components of Different Dissociation Buffers and Their Suitable Samples".
5. Please use the reagent as soon as possible after opening to avoid affecting the results of subsequent experiments.
6. For your safety and health, wear a lab coat and disposable gloves during operation.

Attached Table (Data sourced from published papers, for reference only)

Components and Functions of Different Dissociation Buffers

Components	Functions	Notes	Related Buffers	Dissociation
Magnesium chloride, Magnesium sulfate	Stabilize nuclear chromatin	Magnesium ions	G, T, H, W, m	
Spermine, Spermidine	Stabilize nuclear chromatin	Spermine	L, GP	
Potassium chloride, Sodium chloride	Provide a certain ionic strength and maintain osmotic pressure	Inorganic salts	H, W, L, GP, M	
Triton X-100	Release nuclei, remove cytoplasm, disperse chloroplasts, reduce nuclear adhesion	Surfactant	G, T, H, W, L, m, GP	
Tween	Release nuclei, remove cytoplasm, disperse chloroplasts, reduce nuclear adhesion	Surfactant	O, M	
EDTA	Bind divalent cations serving as nuclease cofactors and inhibit nuclease activity	Metal chelator & substitute	W, L, m, M	
Sodium citrate	Bind divalent cations serving as nuclease cofactors and inhibit nuclease activity	Metal chelator & substitute	G, GP, O, m, M	
Glucose	Maintain nuclear integrity and prevent nuclear adhesion	-	M	
Tris	Maintain nuclear integrity, prevent nuclear adhesion, and stabilize pH	Buffer	T, W, L	
MOPS	Stabilize the pH of the solution	Buffer	G, m, GP	
HEPES	Stabilize the pH of the solution	Buffer	H, M	
PVP	Eliminate the interference of viscous substances such as phenols	-	W, m	

Components	Functions	Notes	Related Buffers	Dissociation
DTT	Protect chromatin proteins and eliminate the interference of viscous substances such as phenols	Reducing agent	H	
2-Mercaptoethanol	Protect chromatin proteins and eliminate the interference of viscous substances such as phenols	Reducing agent	L, m, M	
Sodium metabisulfite	Protect chromatin proteins and eliminate the interference of viscous substances such as phenols	Reducing agent	W	

Components of Different Dissociation Buffers and Their Suitable Samples

Dissociation (Abbreviation)	Buffer	Components	Successfully Tested Samples
Galbraith (G)		Magnesium chloride, Sodium citrate, MOPS, Triton X-100	Pinus, Solanum lycopersicum, Cunninghamia lanceolata
Tris-MgCl ₂ (T)		Tris, Magnesium chloride, Triton X-100	Centaurea cyanus, Celtis australis
HEPES (H)		HEPES, Magnesium sulfate, Potassium chloride, DTT, Triton X-100	Cunninghamia lanceolata
WPB (W)		Tris, Magnesium chloride, EDTA, Sodium chloride, Sodium metabisulfite, PVP, Triton X-100	Papaver rhoeas, Pinus pinea, Prunus domestica, Prunus persica, Pyrus pyrifolia, Quercus robur, Rosa spp., Vitis vinifera, Salix babylonica
LB01 (L)		Tris, Spermine, EDTA, Sodium chloride, Potassium chloride, Triton X-100, 2-Mercaptoethanol	Vicia faba, Dioscorea opposita, Ocimum spp., Picea abies, Solanum melongena, Solanum lycopersicum

Dissociation (Abbreviation)	Buffer	Components	Successfully Tested Samples
mGb (m)		MOPS, PVP, Magnesium chloride, EDTA, Sodium citrate, Triton X-100, 2-Mercaptoethanol	Senecio spp., Arabidopsis thaliana
GPB (GP)		Spermine, Sodium citrate, Potassium chloride, Sodium chloride, Triton X-100, MOPS	Populus euphratica, Populus pruinosa, Ostryopsis davidiana, Pisum sativum, Ipomoea pes-caprae, Allium fistulosum
OTTO (O)		Citric acid, Tween, Disodium hydrogen phosphate	Hippophae spp., Ranunculaceae, Cunninghamia lanceolata
Marie (M)		Tween, Glucose, Potassium chloride, Sodium chloride, EDTA, Sodium citrate, HEPES, 2-Mercaptoethanol	Cunninghamia lanceolata

Specifications

Property	Value
Specifications & Purity	BioReagent
Stability And Storage	Store at -20°C long term (12 months).
Storage Conditions	Store at -20°C
Shipped In	Ice chest + Ice pads. This product requires cold chain shipping. Ground and other economy services are not available.
Grade	BioReagent
pH	7.8±0.1

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