Kiyan Danesh

KiyanZOL

Total RNA extraction reagent
Cat.No.: KEX505

Product information:

KiyanZOL Reagent is a monophasic solution of phenol and guanidine thiocyanate for isolation of high-quality total RNA from cell and tissue samples of human, animal, yeast, and bacteria. KiyanZOL Reagent maintains the integrity of the RNA due to highly effective inhibition of RNase activity while disrupting cells and dissolving cell components during sample homogenization.

Principle of the KiyanZOL:

After homogenizing the sample with KiyanZOL Reagent, chloroform is added, and the homogenate is allowed to separate into aqueous and organic phases by centrifugation. RNA is precipitated from the aqueous phases with isopropanol. The precipitated RNA is washed using %75 ethanol, and then re-suspended in RNA preservation solution for use in downstream applications.

Kit content and storage:

Content	volume	storage
KiyanZol reagent	25 or 50 ml	°4 C*
RNA preservation solution	1 ml	°4 C

KiyanZOL reagent is stable for at least 12 months in °4 C

Sample requirement:

•Perform RNA isolation immediately after sample collection or quick-freeze samples immediately after collection and store at –80 °C or in liquid nitrogen until RNA isolation

Sample type	Starting material/ 1ml of KiyanZOL
Tissue	50 mg
Cells grown in monolayer	1×10^5-1×10^7 cells
Cells grown in suspension	$5–10\times10^6$ cells from animal, plant, or yeast or 1×10^8 cells of bacterial origin

Required equipment and materials not supplied:

1- Centrifuge and rotor capable of reaching 12,000 × g and 4 °C
2- Nuclease-free microcentrifuge tubes
3- Dounce homogenizer or TissueLyser for tissue disruption and homogenization
4- Chloroform
5- Isopropanol
6- Ethanol %75 (diluted by DEPC-treated water)

Procedural guidelines:

•Perform all steps at room temperature (20–25°C) unless otherwise noted.

•Use disposable RNase-free pipette tips, and tubes.

•Wear disposable gloves while handling reagents and RNA samples to prevent RNase contamination from the surface of the skin

•If your sample is cultural cells, do not wash cells before addition of KiyanZOL Reagent to avoid mRNA degradation.

1.samples Homogenization

•**Tissues:** Add 1 mL of KiyanZOL Reagent to 50–100 mg of frozen tissue and homogenize using a homogenizer on ice.

•**Cell grown in monolayer:** discard growth media and add 1 ml KiyanZOL reagent per 1×10^5- 1×10^7 cells directly to the flask or culture dish. Pipet the lysate up and down several times to homogenize.

•**Cells grown in suspension:** Pellet the cells by centrifugation and discard the supernatant.

Then add 1 mL of KiyanZOL Reagent to sample ($5–10\times10^6$ animal, plant, or yeast cells or 1×10^8 cells of bacteria) to the pellet. Resuspend the cells in reagent by pipetting.

Note: After homogenization, samples can be stored at -80°C for at least one month.

2.PHASE SEPARATION:

Optional: If samples have a high fat content, centrifuge the lysate for 5 minutes at 12,000 × g at 4°C, then transfer the clear supernatant to a new tube.

•Incubate the homogenized samples for 5 minutes at room temperature

•Following sample lysis, add 0.2 ml of chloroform per 1 ml of KiyanZOL Reagent. Cap sample tubes securely. Shake tubes **vigorously** by hand for 15 seconds and incubate them at room temperature for 2 to 4 minutes.

•Centrifuge the samples at 12,000 × g for 15 minutes at 4°C.

•Following centrifugation, Sample will separate in 3 layers:

a. Top layer clear aqueous phase = RNA

b. Middle layer white cloudy phase = DNA

c. Bottom layer phenol phase = protein

3.RNA PRECIPITATION:

•Transfer the 80% of upper aqueous layer to a new tube (avoid to transfer the interphase or lower phase)

•Precipitate the RNA by adding 0.5 ml of isopropanol per 1 ml

of KiyanZOL Reagent used for the initial homogenization.

•Incubate samples at -20°C for 30 minutes and then centrifuge at 12,000 × g for 10 minutes at 4°C to precipitate the RNA

4.RNA WASH:

Remove the supernatant and add 1 ml of 75% ethanol to the microtube. Mix the sample by vortexing for 5 sec and centrifuge at 7,500 × g for 5 minutes at 4°C. discard all the supernatant using pipette (Be careful not to disturb the pellet).

Repeat this step again

5. REDISSOLVING THE RNA:

At the end of the procedure, allow air-dry the pellet for 10 minutes at RT and dissolve the pellet in 20- 40 µl RNA preservation solution or RNase-free water

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Troubleshooting:

Problem	Cause	solution
Low yield	The samples were incompletely homogenized or lysed.	Cut tissue samples into smaller pieces and then homogenize using dounce homogenizer. Be sure to incubate for 5 min at room temperature after homogenization
	The pellet was incompletely dissolved in RNA preservation solution	Increase the solubilization rate by pipetting the sample repeatedly. Do not allow RNA pellet to dry completely.
	Too much sample	Decrease the amount of starting material.
RNA degradation	Starting material not handled/stored properly	Sample must be processed or frozen immediately after collection.
	RNase contamination	Use RNase-free tips and microtube
	Frozen tissue thawed in absence of KiyanZOL Reagent.	Add frozen tissue directly to KiyanZOL Reagent
Low OD ratios	Low A280/260 values indicate protein contamination in the sample	Decrease the amount of starting sample
		avoid to draw off the entire aqueous layer after phase separation.
DNA Contamination	Part of the interphase was transferd with aqueous phase	Be sure not to take any of the interphase with the aqueous phase.
	Insufficient KiyanZOL Reagent used	Use 1 ml of KiyanZOL reagent per 50 mg of tissue or 10^6 cells

Note: it is essential to treat RNA samples with DNase before cDNA synthesis or RNA-seq. Failure to treat with DNase or inefficient DNase treatment can affect your results.