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10 Minute DNA Release Kit -7

(10 Minutes Cells Pure DNA Kit)

This Kit was designed to obtain DNA from cells within 10 minutes. It is easily and quickly to have the cell DNA ready for PCR, DNA Methylation, Southern blot and etc. any in the DNA research.

Catalog Number	DNA Release Buffer		Samples	Storage & Use
	Name	Volume		
JZ-007	Kit7-B1	2.5ml	100 or 50 dishes	-Can be kept at 5˚-29˚ C; - Work well for 1 year from the open day.
	Kit7-B2	2.5ml		

Tools

- Thermo-machine or water bath which can be set for a constant temperature between 86˚ - 90˚ C;
- Centrifuge with 10,000 – 15,000g /minute set up at room temperature;
- 1.5ml eppendorf tubes, centrifuge tubes, tips, and micro (10-200ul scalar) pipette;
- Vortex or mixture machine is optional;
- 1 x PBS regent (see instructions in the table below).

Making 1x PBS, ph 7.3±, without Ca++ and Mg ++

Reagent	Final Concentration	For 1000 ml
NaCl	137mM	8.0g
KCl	2.7mM	0.2g
Na2HPO4-7H2O	4.3mM	1.15g
KH2PO4	1.4mM	0.2g
Dissolved in 1000ml of Distilled Water		

Work Table

Step	Action
	Preparing of Cells' Pellet from 50,000-1,000,000 Cells
Step #1	• Place, trypsinized and harvested with growth media, cells to a 15ml centrifuge tube. • Centrifuge them at 1000rmp for 5min. • Remove supernatant and leave the cells' pellet at the bottom of the tube.
Step #2	• Add 1ml of 1 x PBS to the tube to re-suspend the cells pellet. • Transfer the cells pellet to a clean eppendorf tube. • Centrifuge it at 6000rpm for 3min. • Decant the PBS leaving the pellet at the bottom of the tube.
	Release of DNA
Step #3	• Add **25ul of Kit7-B1** to the tube containing cells' pellet. • Flick it 3-5 times to suspend it.
Step #4	• Place the tube in a thermo-machine or a water bath at 86˚-95˚ C for 8min (no shaking); • Remove the tube from the thermo-machine to RT. • Flick the tube 3-5 times again.
Step #5	• Add **25ul of Kit7-B2** to the tube. • Flick the tube again. • Centrifuge it at 15,000g for 2 min. at RT.
Step #6	• Transfer 40-50ul of clear aqueous phase which is the DNA extract into a clean tube • The sample is ready to run PCR or Southern (for Southern measure its ratio and concentration).

Preparing of Cells' Pellet and Releasing DNA from 100mm Cells Culture Dish

The processing is same in step 1, 2 and 4

- Use **50ul** of Kit7-B1 in **Step #3**
- Use **50ul** of Kit7-B2 in **Step #5**
- Transfer **80-90 ul** of DNA extract in **Step #6**.

Disclaimer: There is no guaranty of getting a better quality DNA extract if using scraped cells.

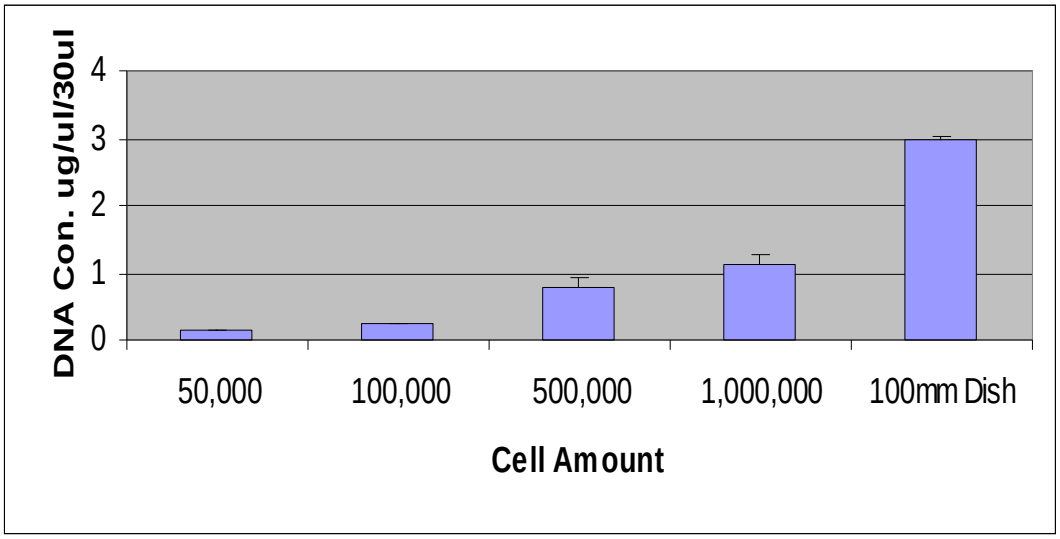
Notes:

- Use 1-3ul of DNA extract to run PCR at 20-25 reaction volume.
- Using the kit for the first time, determine the best working range of the extract by running PCR with different quantities of 1ul, 2ul, and 3ul of DNA.

Correlation between DNA Extract Quantity and Number of Cells

Cells	Concentration (ug/ul)		Volume/Each	Ratio on 260/280 nm
	Range	Average		
5 X 104	0.12-0.16	0.14	32-36 ul	1.7-2.0
1 X 105	0.22-0.26	0.24	32-36 ul	
5 X 105	0.7-0.9	0.8	32-36 ul	
1 X 106	0.9-1.5	1.2	32-36 ul	
100 mm cell culture dish	1-1.6	1.3	70 ul	
	2.93-2.99	2.96	30 ul	

Illustration of Correlation between DNA Extract Quantity and Number of Cells



Order Price

Name	Cat.#	Price/Kit	Assays
Ten Minute DNA Release Kit-7	JZ-007	$ 68. 50/1 kit	100

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