نشانی: تهران خیابان پاسداران میدان اختیاریه، خیابان شهید زرنگ، ابتدای خیابان شهید دوقوز ساختمان نگین طبقه٤ واحد ١٢ تلفن:1 -٢٢٧٦٢٧٠٠ فاکس : ٢٢٧٦٢٧٠٢

No.12, Negin building, Doghouze St., Zerang St., Ekhtiarieh Sq. Pasdaran St., Tehran

Tel: 021 22762700-1, Fax: 22762702

www.pgazma.com

شرکت تحقیقاتی تولیدی پویاژن آزما

POUYA GENE AZMA Co. (PGA)

PGA DNA Extraction kit

for Gram-Negative Bacteria

Catalog No. PD110-050
Quantity: 50 Preps

Store at: RT

For research use only

KIT CONTENTS:

Buffer I	5 ml
Buffer II	10 ml
Buffer X	10 ml
RNase A	
Solvent Buffer	10 ml

Need contents :

cold ethanol %100
cold ethanol %70

TROUBLESHOOTING

	Possible reason	Solution
Low Yield of DNA	Low precipitatio. Incomplete lysis	Take new sample. Keep in 37oC for more time
smear DNA bands	Improper electrophoresis conditions were used.	Do not allow voltage to exceed ~20 V/cm. Maintain a temperature <30° C during electrophoresis. Check that the electrophoresis buffer used had sufficient buffer capacity. This is done by checking the pH in the anode and cathode chambers.
	There was too much salt in the DNA.	Use ethanol precipitation to remove excess salts, prior to electrophoresis.

Kit Description:

This kit contains all ingredients for quick preparation of pure DNA from gram negative bacteria. This kit presents remarkable features of timesaving, easy, prompt and high yield DNA purification. The procedure requires 30 minutes and does not require enzymes or phenol extraction . DNA obtained by this method can be used for all molecular biology procedures (PCR, restriction digestion, cloning, Southern blot, DNA sequencing, etc.).

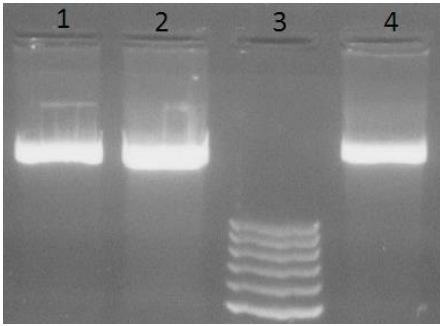

Fig.1: Extracted DNA by PGA DNA Extraction kit from gram negative bacteria on %1 agarose

1. E. coli 2**.** *Psedomonas* 3. marker

4**.** *Citrobacter*

Attention: before use , please add RNase A to Buffer I and keep in 4 ºC

LABORATORY PROTOCOL:

1: **Collect 1 - 2 ml gram-negative bacterial cultures by centrifugation 13000 rpm for 1 min in microtube.**

2: **Resuspending of precipitate in 100 µl Buffer I.**

3: **Add 200 µl Buffer II in tube.**

4: **Add 180 µl Buffer X in tube and inverting for 10 times (15 Seconds).** (when add X buffer in tube and inverting , producing white precipitate in microtube)

5: **Microcentrifuge 13000 rpm for 10 minutes.**

6: **Transfer of supernatant in new tube.**

(important: do not transfer of precipitate in new tube. If transfer of precipitate in it please repeat 9 and 10 steps again)

7: **Add 2 volumes cold ethanol %96 - %100 in solution and gently inverting for 5 times.**

8: **Microcentrifuge 13000 rpm for 5 minutes.**

9: **Pour off the ethanol by gently inverting of tube and keep precipitate.**

10: **Washing the precipitate by adding 700 µl cold ethanol %70 and inverting 2-3 times.**

11: **Microcentrifuge 13000 rpm for 1 minute.**

12: **Pour off the ethanol completely and dry pellet for 1-2 minutes in room temperature.**

13: **According to precipitate add 20 - 50 µl Solvent Buffer in tube. The precipitate must to be solve completely.**