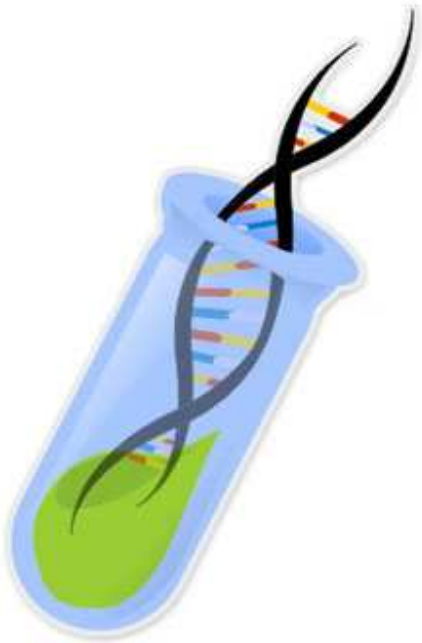
Research Center for
Pharmaceutical Nanotechnology

General DNA Extraction Kit

(*TabDNA Kit*)

INSTRUCTION MANUAL



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Introduction:

General DNA Extraction Kit provides high yields of exceptionally pure DNA from a wide variety of living organisms. General DNA Extraction Kit is especially designed to provide high yields of DNA from moderately and highly processed samples. The extracted and purified DNA is suitable for any molecular biology procedure, such as PCR (Polymerase Chain Reaction) amplification, restriction digestion, cloning, sequencing, etc. We recommend the use of General DNA Extraction Kit for optimal PCR-based amplification and minimal inhibition. General DNA Extraction Kit is able to extract DNA from different varieties of sample. The General DNA Extraction Kit works on whole living organisms such as bacteria (gram + and -), fungi, plant and animal cells. General DNA Extraction Kit contains powerful denaturants and DNA-selective reagents that are specifically designed to prevent DNA degradation and to remove contaminants that might inhibit the PCR reaction or generate artifactual DNA template-primer interactions.

Kit Components:

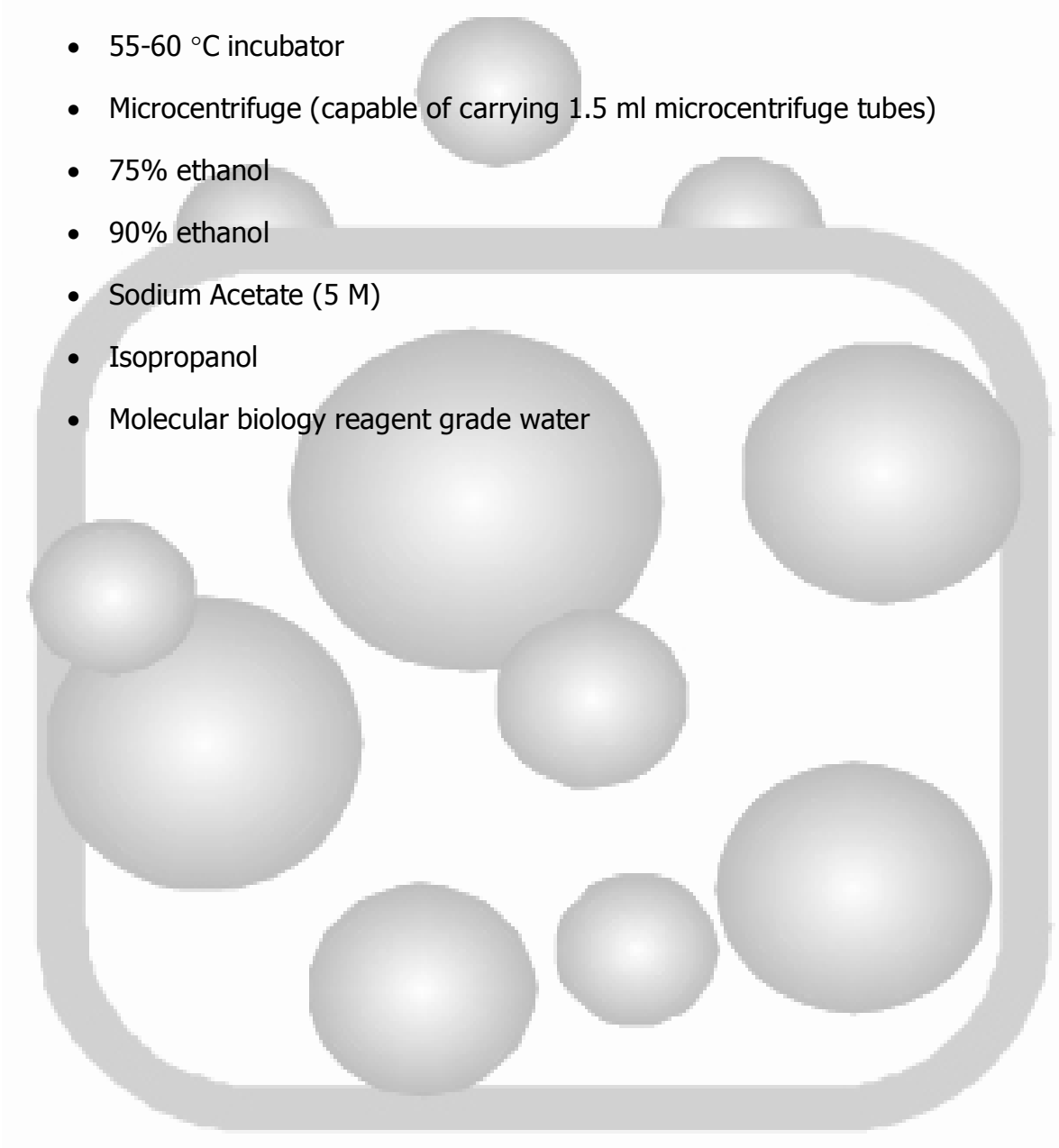
Kit Components	Preparations
Buffer BL	**100 ml**
Buffer GE	**100 ml**
Chloroform- isoamilalcohol (24:1)	**100 ml**
1xTE (10 mM Tris-HCl, 1 mM Na-EDTA pH 8.0)	**100 ml**

Storage Conditions:

All components of the General DNA Extraction Kit should be stored at room temperature (20-25 ̊C) and are stable for one year under these conditions.

Recommended Equipment and Reagents (not supplied):

- Liquid Nitrogen
- Mortar and pestle
- 55-60 °C incubator
- Microcentrifuge (capable of carrying 1.5 ml microcentrifuge tubes)
- 75% ethanol
- 90% ethanol
- Sodium Acetate (5 M)
- Isopropanol
- Molecular biology reagent grade water



Introduction to the Protocol

Unlike many DNA extraction kits, General DNA Extraction Kit is applicable to a wide range of sample types. The protocol that follows provides specific variations for extracting DNA from different sample types in order to maximize yield, concentration and quality of the extracted DNA. Through experimentation, DNA from various sample types can be successfully extracted with General DNA Extraction Kit.

General Overview of the Protocol

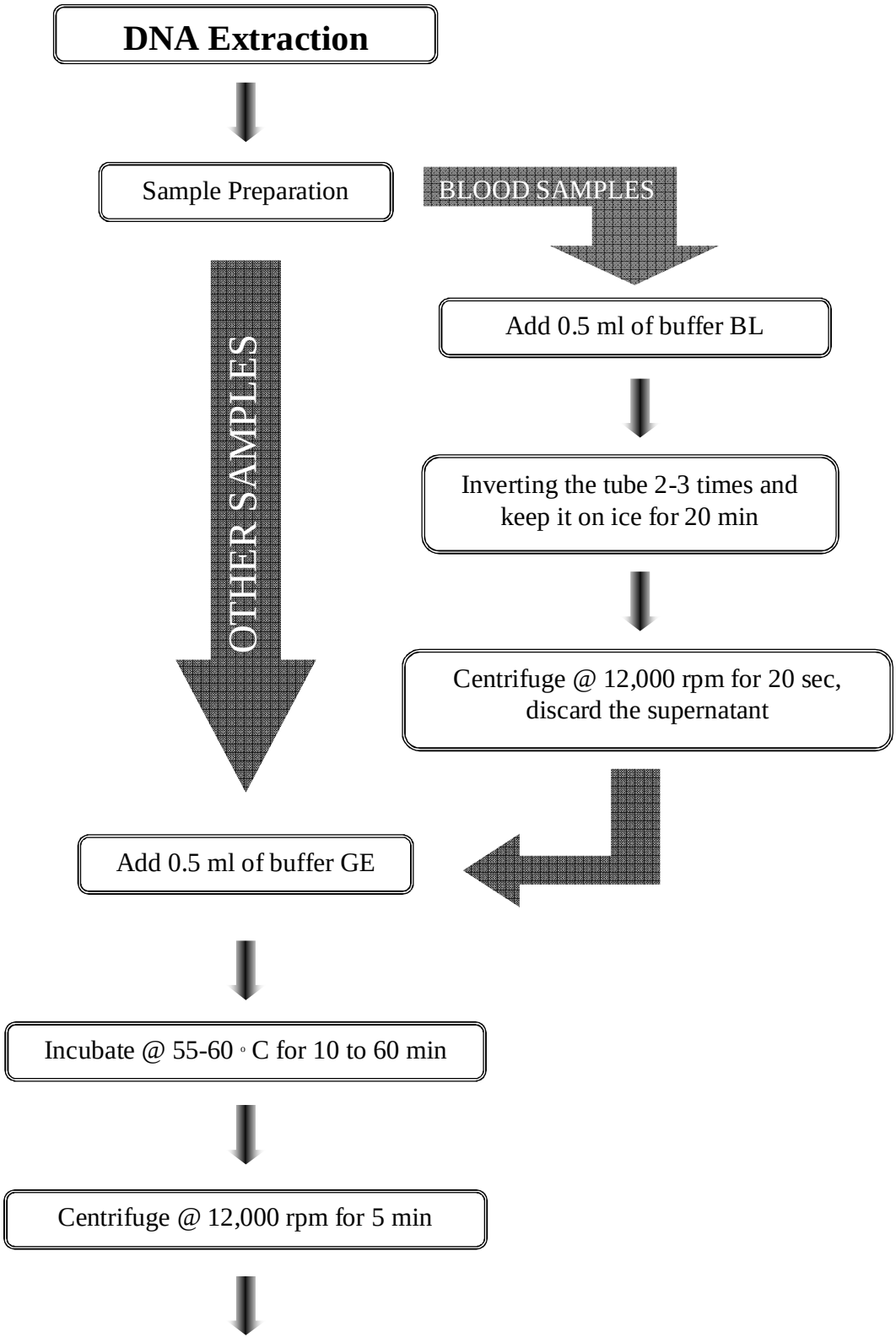
1. Prior to using the protocol, we recommend that care be taken in homogenizing the sample in a manner that minimizes the risk of inadvertently contaminating the sample. Contamination can occur by not using properly cleaned equipment or using poor laboratory practices during homogenization, weighing and labeling of the subsample and/or archiving of the remainder of the sample.

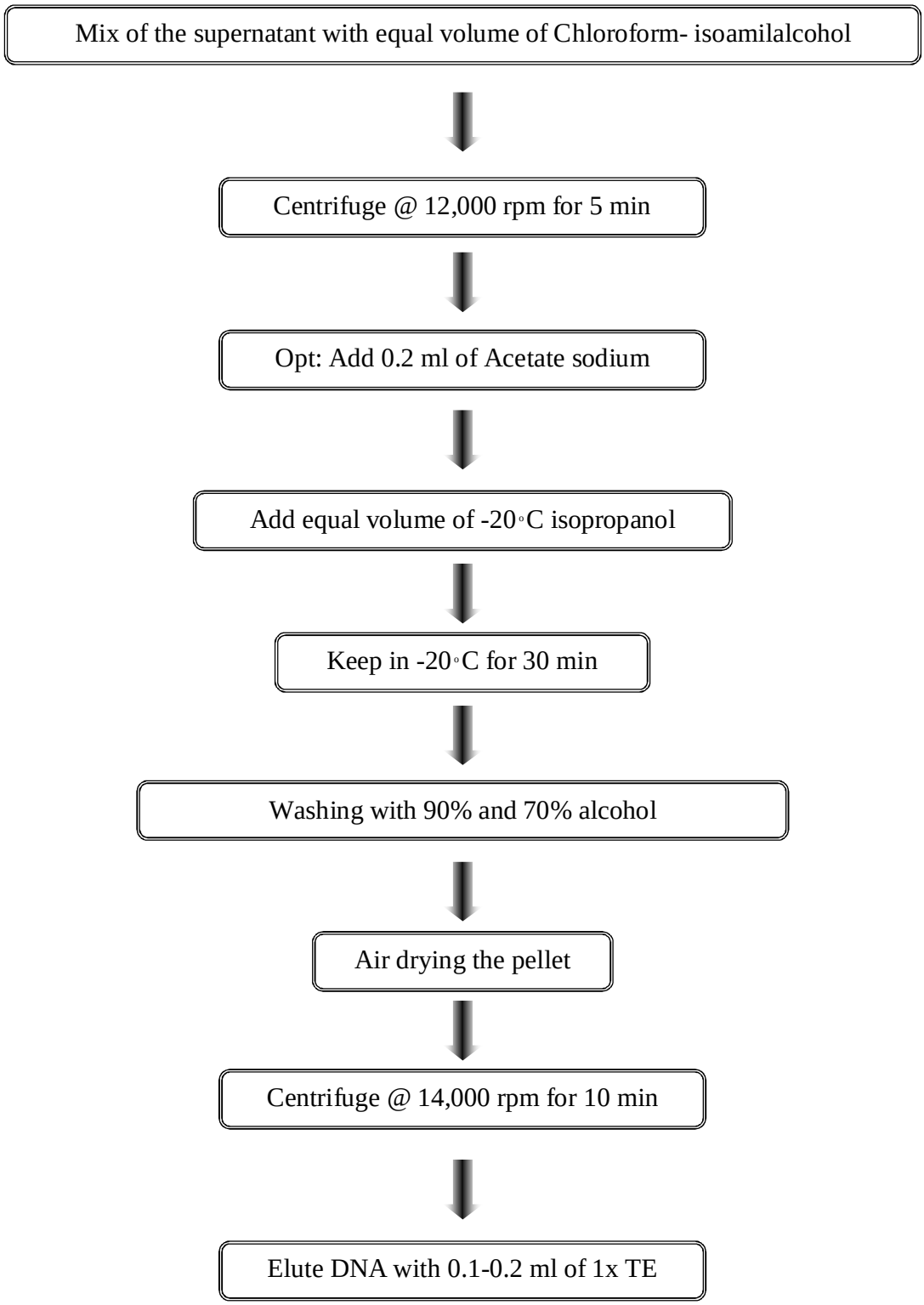
2. Buffer GE is a main buffer would be used for extraction of DNA from the all samples which lyses the cells of the subsample and solubilizes proteins, DNA, and other cellular constituents.

3. Buffer BL is being used exclusively in process of blood samples in addition to buffer GE.

5. DNA is dissolved in 1xTE and is ready for use.

6. For optimal results it is necessary to use the reagents provided with the kit, except for those listed above.


DNA Extraction
Sample Preparation
BLOOD SAMPLES
OTHER SAMPLES
Add 0.5 ml of buffer BL
Inverting the tube 2-3 times and keep it on ice for 20 min
Centrifuge @ 12,000 rpm for 20 sec, discard the supernatant
Add 0.5 ml of buffer GE
Incubate @ 55-60 ◦ C for 10 to 60 min
Centrifuge @ 12,000 rpm for 5 min


Mix of the supernatant with equal volume of Chloroform- isoamilalcohol
Centrifuge @ 12,000 rpm for 5 min
Opt: Add 0.2 ml of Acetate sodium
Add equal volume of -20◦C isopropanol
Keep in -20◦C for 30 min
Washing with 90% and 70% alcohol
Air drying the pellet
Centrifuge @ 14,000 rpm for 10 min
Elute DNA with 0.1-0.2 ml of 1x TE

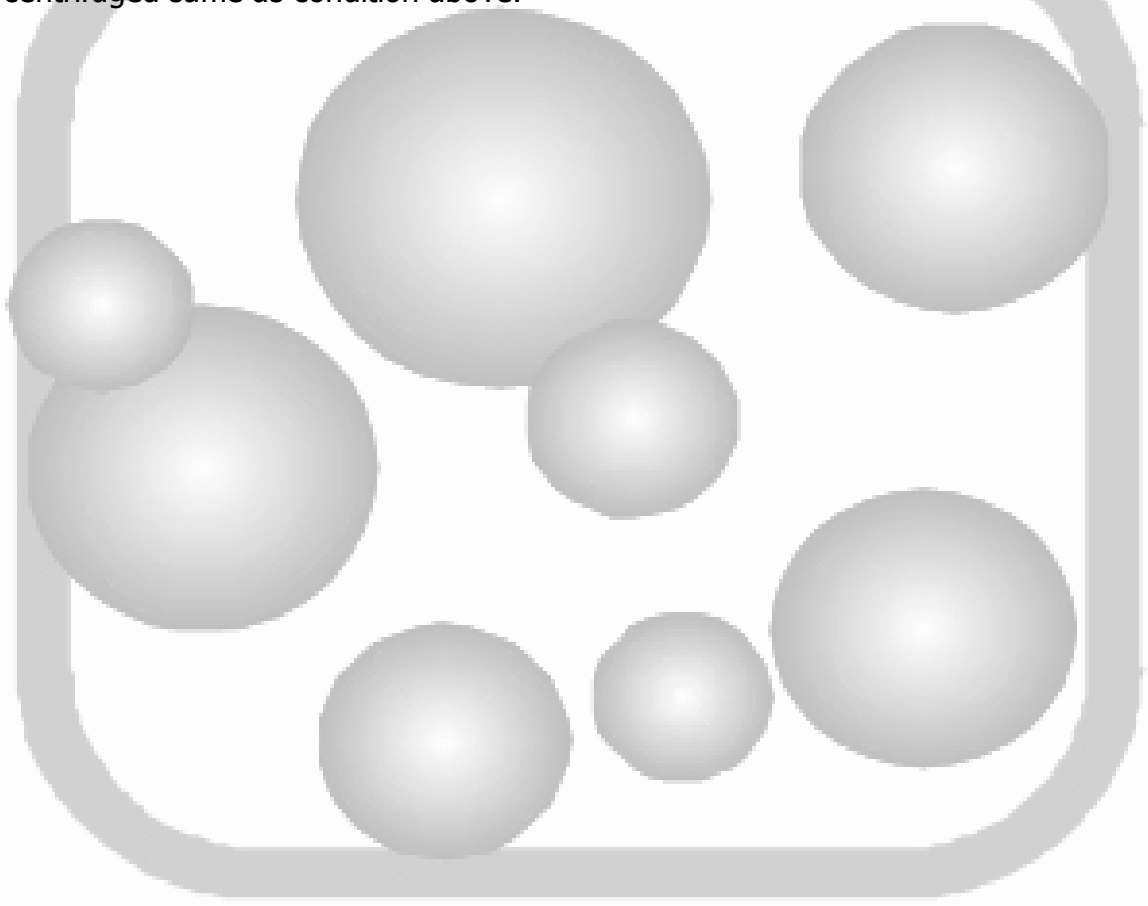
DNA Extraction Procedure

Sample preparation:

Solid samples such as fungi, colonies of bacteria, plant or animals tissue would be pulverized with liquid nitrogen using mortar and pestle.

Liquid samples such as bacterial liquid media; etc. need to be centrifuge at 12,000 rpm for 10 min to have the desired amount of pellet. Then tube would be shocked in liquid nitrogen and 60 C incubator for 2-3 times.

Blood samples don't undergo liquid nitrogen process but need to be centrifuged same as condition above.



DNA Extraction

1. 500 of buffer GE were added preprepared sample in 1.5 eppendroff tube

Note: Blood samples before this step must be treated with buffer BL with the condition below:

- Add 0.5 ml of buffer BL
- Inverting the tube 2-3 times and keep it on ice for 20 min
- Centrifuge at 12,000 rpm for 20 sec, discard the supernatant

2. Incubation of sample at 55-60°C for 60 min (for gram - bacteria 10 min is adequate)
3. Centrifuging sample at 12,000 rpm for 5 min
4. Adding equal volume of Chloroform- isoamilalcohol to the supernatant
5. Tube was centrifuged at 12000 rpm for 5 min then transferring supernatant to a new tube

Optional: Adding 0.2 ml of Acetate sodium in this stage will excess the quality of extracted DNA

6. Add equal volume of -20 °C isopropanol
7. Keeping sample in -20 °C for 30 min
8. The tube was centrifuged at 13000 rpm for 10 min
9. The supernatant was discarded and 500 µl of 4°C 96% ethanol was added
10. The tubes were centrifuge at 13000 rpm for 10 min, the supernatant was discarded and 500 µl of 4°C 70% ethanol was added.
11. The tube was centrifuged at 13000 rpm for 5 min to stick the pellet at the bottom of tube.
12. The supernatant was discarded and the pellet was let to be dried at room temperature.
13. The pellet was dissolved in 50 µl of TE or sterile distilled water.

Technical Assistance and Ordering Information

For technical assistance or ordering information refer to our website on http://nano.tbzmed.ac.ir, contact pharm@sinaatashpaz.com or call +98 914 316 7108.

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