Jul 26, 2022

Preparing 10x PCR buffer

In 1 collection

Nadine Mowoh[1], Stephane Fadanka[1]

[1]Beneficial Bio, Mboalab

1 *Works for me* Share

This protocol is published without a DOI.

Low-cost, high-quality ...

Nadine Mowoh

ABSTRACT

10X ThermoPol Reaction Buffer is optimized for use with Vent® and Deep Vent® DNA Polymerases. This buffer also provides superior reaction conditions for other thermophilic DNA polymerases, including *Taq*DNA Polymerase, OpenVent as well as various other DNA and RNA modifying enzymes.

Here we describe the protocol used in making a 10x PCR buffer recipe that provides optimum reaction condition for OpenVent polymerase enzyme.

10x PCR buffer comprise of:

100 mM KCL
100 mM (NH)2SO4
200 mM Tris-HCL pH8.8 @ 25c
20 mM $MgSO_4$
1 % Triton x100

PROTOCOL CITATION

Nadine Mowoh, Stephane Fadanka 2022. Preparing 10x PCR buffer. **protocols.io** https://protocols.io/view/preparing-10x-pcr-buffer-cc79szr6

COLLECTIONS

Beneficial Bio Products

KEYWORDS

10x PCR buffer recipe, Composition of 10x PCR buffer

CREATED

Jul 12, 2022

LAST MODIFIED

Jul 26, 2022

PROTOCOL INTEGER ID

66529

PARENT PROTOCOLS

Part of collection
Beneficial Bio Products

GUIDELINES

This protocol describes the steps in preparing a low cost 10x PCR buffer.

MATERIALS TEXT

Chemicals

Tris **Roche Diagnostics**

Hydrochloric acid **Thermo Scientific**

Potassium chloride **Fisher Scientific**

Ammonium sulphate **Sigma Aldrich**

Magnesium sulphate **Sigma Aldrich**

Triton X-100 **Fisher Scientific**

Distilled Water **Contributed by users**

Materials and Equipment

Sterile Pipette tips (P-10, P200, P-1000)
Micropipettes
Sterile falcon tubes
Sterile Eppendorf tubes

SAFETY WARNINGS

- HCL is very corrosive and should be handled with care.
- Wear protective clothing (Lab coat), face masks and gloves.

BEFORE STARTING

Make sure all chemicals are available in their right concentrations needed to make the 10x PCR buffer.

Preparing reagent stocks

1 ⚠

Before preparing the 10x PCR buffer, prepare 1M and initial stocks of the different chemicals that would be used.

- We adopt 3 M stock of HCL to use in preparing Tris-HCL
- Prepare 10 mL of 3 M HCL by diluting 3.129 mL of concentrated or 37% HCL in 6.871 mL of distilled water.

⚠ When you mix acid with water, it's extremely important to add the **acid to the water** rather than the other way around. This is because acid and water react in a vigorous exothermic reaction, releasing heat, sometimes boiling the liquid.

Prepare 10 mL of 1 M concentration of each of the chemical stocks by weighing the salt powders as indicated in the table 1 below:

- Weigh out the salts as indicated in the table and add to a 100 mL glass beaker.
- Measure out 10 mL of distilled water and add to the glass beaker.
- Add a magnetic flea and place on a magnetic stirring plate to mix the solution as shown in figure 1
- To sterilize, autoclave the solutions by using a steam autoclave at 121° C for at least 30 minutes under at least 15 psi. *(All the solutions should be transferred into Duran bottles that can withstand heating).*

A	B
Chemical	**Amount needed in (g) for 1 M stock**
Tris	1.211
KCL	0.74
NH(SO4)2	1.30
MgSO4	1.20

Table 1

To make 1M Tris HCL pH 8.8

1. Weigh out **1.211 g** Tris and add to a 50 mL glass beaker.
2. Measure out 8 mL of distilled water and add to the glass beaker.
3. Add a magnetic flea and place on a magnetic stirring plate to mix the solution as shown in figure 1.
4. Add a pH meter into the solution to observe the pH.
5. Slowly add the 3 M hydrochloric acid (HCl) solution using a Pasteur pipette to reduce the pH to **pH 8.8**. Be careful not to add too much at a time, since the pH will change rapidly.
6. Once the desired pH has been reached, top up the solution to 10 mL using distilled water.

Figure 1

Pipetting to make 10x PCR buffer - 1mL

2

Pipette the following reagent stocks into a clean 1.5 mL Eppendorf tube as described in the table below to compose 1 mL of 10x PCR buffer.
1M KCL
1M (NH)2SO4

1M Tris-HCL pH8.8 @ 25c
1M $MgSO_4$
Triton x100

A	B
Chemical stock	**Amount required in uL to make a 1000 mL of 10x PCR Buffer**
1M KCL	100
1M (NH)2SO4	100
1M Tris-HCL pH8.8 @ 25c	200
1M MgSO4	20
Triton x100	10

Table 2

- Cork the tube and invert several times to mix the solutions
- Label the tube appropriately and store at -20c.

- Before using the buffer, carryout a functionality and Nuclease test to show that the buffer formulation is able to provide a suitable PCR condition for DNA amplification and also free from contaminating nucleases.
- We typically use our internal quality control protocol to check this.