COMPLETE TEACHING KIT FOR PCR AMPLIFICATION
50 rxns of 50 µl OR 100 *rxns of 25* µl

Cat No.110232

DATA-SHEET

Polymerase Chain Reaction **(PCR)** is the most powerful technique in molecular biology for amplification of DNA. By PCR one Copy of DNA can be amplified to billions of copies & can then be detected by various methods. We here provide a complete teaching kit for PCR amplification. This kit is specially designed for the Lecturers/Teachers to teach the students about the basic PCR amplification & beginners in the area of Molecular Biology. The kit provides an easy, optimized, PCR assay. The protocol described below is for 50µl reaction volume. In case 25µl reaction Volume is to be used then the Volumes can be just halved for the amplification reagents as shown in the table.

Contents of the Pack:

Item	Quantity
– Taq DNA Polymerase	125 U
– 10 X PCR Buffer	250 µl.
– Magnesium Chloride Sol.	0.5 ml
– dNTP Mix 40 mM	50 µl
– Mol. Grade Water	1.5 ml
– Lamda Primer Forward	100 µl
– Lamda Primer Reverse	100 µl
– Lamda DNA High Conc.	430 µl
– Lamda DNA Low Conc.	430 µl
– 100 bp DNA Mol Weight Marker	60 µl
– Gel Loading Dye	300 µl
– Mineral Oil	1.4 ml.
– Ethidium Bromide	50 µl
– 50 X TAE Buffer	100 ml.
– 0.2 ml PCR Tubes	50 Nos.
– Agarose 25 gms.	1 bottle.
– Pack Insert	1 Nos.

Storage of the Kit: ***Box No. 1 to be stored at*** **-20°C. & Box No 3 to be stored at Room Temp alongwith Box No. 2.**

Manufactured and Marketed By :
GENOME DIAGNOSTICS PVT. LTD.,
(An ISO 13485:2003 & ISO 9001:2000 Certified Company)
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Preparation of Reagents:

All reagents are ready to use except for the following & their preparation is described below.

1 X TAE Buffer: To prepare 1 X TAE Buffer from 50 X dilute the provided 50 X TAE Buffer 1:50 with deionized water. i.e. 1ml of 50 X TAE buffer into 49 ml of Deionized Water.

Precautions:

- The UV in the laminar flow be put on before the usage of laminar flows at least for one hour.
- Turn off the UV at least 5 minutes before the usage of the Laminar flow.
- Put the laminar blower and Fluorescent lamp on.
- The work area should be set in a manner so that there is no hindrance in working.
- Thaw all components thoroughly at room temperature before starting.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice or in the Cooling Block.
- Use pipette tips with filters only.
- Always use disposable powder-free gloves
- Avoid cross contamination
- Spilled droplet must be cleaned with Hypo
- Always discard all used tips etc. in the Hypo.

Protocol

Preparation for amplification:

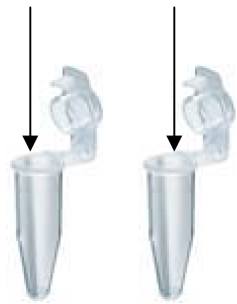
	50 **µl reaction Volume**		***25*** **µl reaction Volume**	
	For 1 reaction	**For 5 reactions**	**For 1 reaction**	**For 5 reactions**
10 X PCR buffer	5.0 µl	25	2.5 µl	12.5
dNTP's Mix	1.0 µl	5 µl	0.5 µl	2.5 µl
$MgCl_2$	4.0 µl	20 µl	2.0 µl	10 µl
Lambda Primer Forward	2.0 µl	10 µl	1.0 µl	5 µl
Lambda Primer Reverse	2.0 µl	10 µl	1.0 µl	5 µl
Taq DNA polymerase	0.5 µl	2.5 µl	0.25 µl	1.25 µl
Water	15.5 µl	77.5 µl	7.75 µl	38.75 µl
Total Volume	30 µl	150 µl	15 µl	75 µl

Protocol for *50* µl reaction Volume

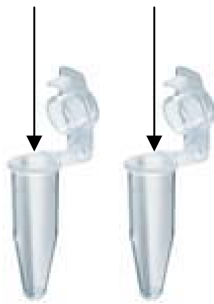
-- Place the 0.2 ml PCR tubes provided in this kit in the PCR rack (Not provided in kit) & dispense 30 µl of the above mixture in each PCR tube.

-- Dispense 20 µl of Low Conc. Lamda DNA (in two tubes) & High Conc. Lamda DNA (in two tubes) & 20 µl of Mol Grade water in one tube as described below.

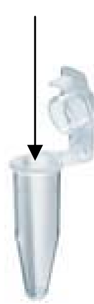
20 µl of Low Conc. Lamda DNA



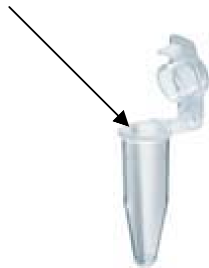
20 µl of High Conc. Lamda DNA



20 µl of Mol. Grade Water.



NOTE: In case of Hot Lid thermal Cyclers there is no need for adding Mineral oil. In case the Thermal Cycler is not having Hot Lid facility then please overlay 25 µl of Mineral oil in each PCR tube by adding the Mineral oil through the sides of the PCR tube as depicted below.



Close the cap of the PCR tubes & load these 0.2ml tubes in the PCR machine and set the cycling conditions as follows:

Initial denaturation : 95^{0} C for 5 min

Cycling:

Denaturation : 95^{0} C for 30 sec
Annealing : 55^{0} C for 30 sec
Extension : 72^{0} C for 30 sec
} For 40 cycles

Final extension : 72^{0} C for 5 min

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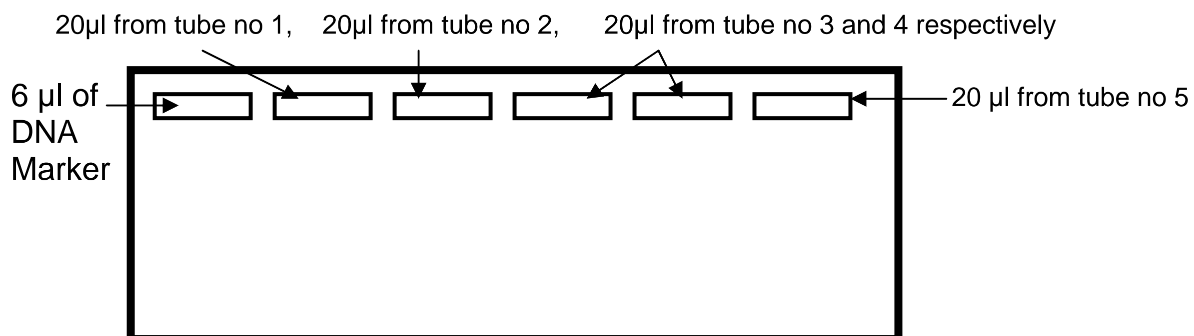
After PCR, perform Agarose gel electrophoresis as described below:

1. Add 1 ml of 50 X TAE buffer to 49 ml of distilled water in order to obtain 1X TAE buffer as described in Preparation of reagents.
2. Add 600 mg of Agarose (provided one pouch) to 50 ml of freshly prepared 1X TAE buffer in a conical Glass flask.
3. Boil the mixture to obtain a clear Agarose solution. Alternatively it can be boiled in a microwave oven as well. As soon as the Sol is clear stop boiling.
4. Add 2 µl of ethidium bromide solution to the agarose solution & mix gently.
5. Pour this solution into a clean gel-casting tray, which has already been sealed from two open sides for the casting of Agarose gel. Place the combs for the formation of the wells in appropriate position immediately after pouring the agarose solution.
6. Allow the mixture to cool for 20 – 30 min to form a gel.
7. After the formation of gel, remove the comb and the edge sealing & transfer the gel to the Electrophoretic gel tank.
8. Fill the electrophoresis gel tank with 1X TAE buffer prepared as mentioned in Preparation of reagents. (Total volume of 1 X TAE to be poured depends on the capacity of the Gel Tank, which could be between 750 ml to 1.5 liters. Please refer to the manual of your Gel Electrophoresis system for confirming the tank volumes)
9. Add 10 µl of the gel loading dye (provided) to the PCR mixture in each tube, which was obtained after PCR amplification. Mix them thoroughly. (In case Mineral oil has been overlaid then remove the mineral oil first with the help of a micropipette & then add & mix the Gel loading Dye in the PCR tubes.
10. Load 6µl of the marker (DNA Ladder – provided) in the first well of the Agarose gel.
11. Load 20 µl of the PCR product mixed with gel loading dye from above tubes from the second well in the Agarose gel onwards as depicted below.



12. Apply 70 – 80V for 45 minutes-1 hour.
13. After the electrophoresis, transfer the gel on to the Transilluminator.
14. View the gel at 260/280 nm.
15. You must be able to observe the bands ladder in the first lane, and a faint band of 277 bp in lane no. 2 & 3, & a bright band of 277 bp in lane no 4 & 5 & no band in lane no 6 which confirms the success of PCR procedure as shown below.

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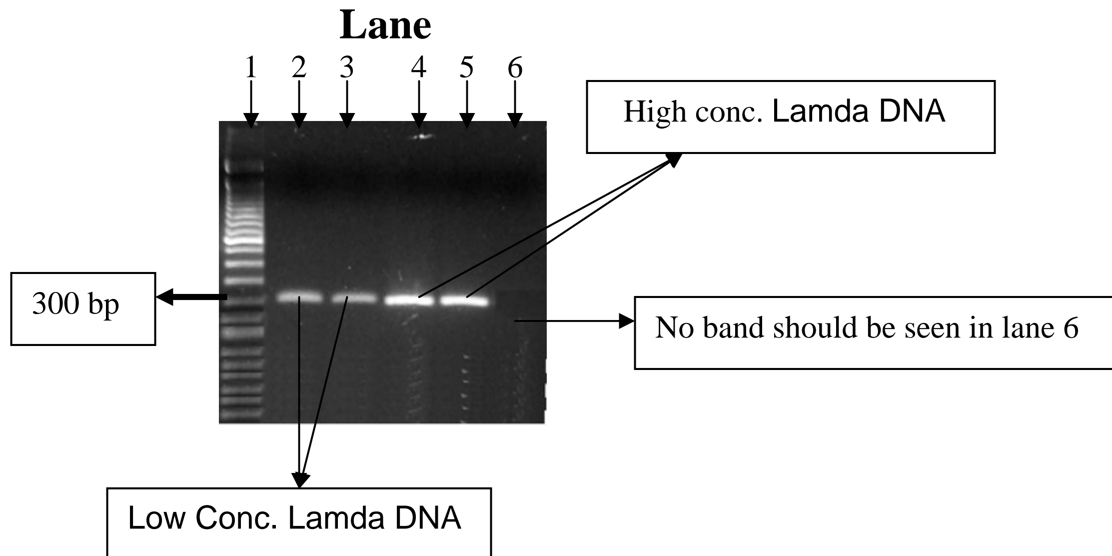

Special Note:

1. **The TAE Buffer once prepared can be Used for at least 5 subsequent runs. Please store the same in a bottle after the run.**
2. **The Lamda DNA & primers have been provided for the Convenience of Usage. However if you wish to your use your own Primers and DNA then it can simply be replaced by the Lamda Primers and DNA and Lamda controls.**

NOTE : For research use only. Not for use in Diagnostic or therapeutic procedures.
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