

Cancer Biology PCR Teacher Preparation

Reagent Preparation for Cancer Biology Kit PCR

- Assumes 12 patient samples tested in duplicate (24 samples total)
- Assumes students work in 8 groups of 4 – each group examining 3 patient samples

2X OneTaq Master Mix

Students will use 12.5 ul of 2X OneTaq Master Mix per reaction for a total of 5 reactions per group: 3 patient samples, 1 positive control reaction and 1 negative control reaction.

Note: 2X OneTaq Master Mix from New England BioLabs (NEB) comes in two formats: with and without loading dye. This protocol assumes that students are using 2X OneTaq Master Mix *without* loading dye.

Example:

- Each student group will need $12.5\text{ul} \times 6 = 62.5\text{ul}$. Give each group an aliquot of 75 ul.
- $8\text{ groups} \times 75\text{ul} = 600\text{ul}$ 2X OneTaq Master Mix is needed.

Primer Mix

The primer mixture contains only the forward primer at a concentration of 10 uM in the mixture. Students will use 2.5 ul of the primer mix for each PCR reaction.

Example:

- Each student group will need $2.5\text{ul} \times 5 = 12.5\text{ul}$. Give each group an aliquot of 15ul.
- $8\text{ groups} \times 15\text{ul} = 120\text{ul}$ of Primer Mix is needed.

DNA Samples

'Patient' Samples

The patient samples contain plasmid DNA at approximately 2.0 ng/ul plus the appropriate reverse primer at a concentration of 2.5uM (to give the band of the desired size). There are three possible band sizes:

- 1,000 kb – one copy of the HER2 gene Labeled 'DNA +1'
- 2,000 kb – two copies of the HER2 gene Labeled 'DNA +2'
- 3,000 kb – three copies of the HER2 gene Labeled 'DNA +3'

Students will use 10 ul of the appropriate DNA mixture for each PCR reaction. Of the 12 patient samples, there are 9 samples that are positive for the 1,000 kb band. There is one patient sample that is positive for a 2,000 kb band. There are two patients that are positive for a 3,000 kb band. Remember, each patient sample is tested in duplicate. Consult the 'Patient Key' to determine which patient samples should be positive by PCR and how to label these tubes. Add 10 ul of the appropriate DNA solution to each of these sample tubes.

Example:

- 9 (DNA +1) positive samples x 2 (sample duplicates) x 10 ul = 180 ul of the "DNA +1" DNA sample

PCR Positive and Negative Controls

Each student group will set up a positive control PCR reaction and a negative control PCR reaction.

- **Positive control (labeled "Pos"):** Each student group will use 10 ul of the plasmid DNA mixture in their positive control reaction.
 - The HER2 (DNA +2) sample is used as the positive control.
 - *Example:*
8 PCR positive control samples x 10 ul = 80 ul of the DNA mixture.
- **Negative control (labeled "Neg"):** Each student group will use 10 ul of sterile distilled water in their negative control reaction. Add 10 ul of sterile distilled water to each PCR negative control sample tube.
 - *Example:*
8 PCR negative samples x 10 ul = 80 ul of sterile distilled water.

6X Loading Dye

Each student group will add 4 ul of 6X Loading Dye to each of their 5 PCR reactions before loading them on a gel.

Example:

- Each student group will need 4 ul x 5 = 20 ul of 6X loading dye. Give each group an aliquot of 30 ul.
- 8 groups x 30 ul = 240 ul of 6X Loading Dye is needed

1kb DNA Ladder

Each student group will need 12 ul of the 1kb DNA ladder to load on their gel.

Example:

- Each student group will need 12 ul. Give each group an aliquot of 15ul.
- 8 groups x 15 ul = 120 ul of the 1kb DNA Ladder is needed

1 X TAE

The TAE is supplied as a 50X solution (50 times more concentrated than what it should be used at). It must be diluted to 1X before use.

Example:

- To make 1 liter of 1X TAE, add 20 ml of the 50X TAE to 980 ml of distilled water.
- Mix well before using.

Ordering information/amounts from New England Biolabs (NEB) for the Cancer Biology PCR Lab:

All other reagents are obtained from Shoreline Biotech Experience Staff as part of the Cancer Biology Kit.

1. 2X OneTag Master Mix – This reagent (M0484S) comes in a volume of 1.25 mL. It is used at 75 ul (62.5uL plus extra)/student groups (8 student groups). So, one vial can support 2 classes investigating 12 patient samples in duplicate (3 samples + 2 controls / 8 student groups, each group containing 4 students).
2. Quick Load 1kb DNA ladder – This reagent (N0468S) comes in a volume of 1.25 ml. Students load 12 ul on each gel. One vial can support 100 gels. Each student group runs one gel.
3. Gel Loading Dye, Purple (6X), no SDS – This reagent (B7025S) comes in a volume of 4 ml (4,000 ul). Student groups use 4ul x 5 reactions = 20 ul. One vial can support 200 student groups.

Note:

All of the above reagents last for at least 2 years if properly stored at –20°C degrees (i.e., frozen).

To order these items from NEB, you must:

1. Register for an educators account by completing the form at:
<https://www.neb.com/promoting-science-education/course-support-and-reagent-donation>

2. Email you order to the email address on the Educational Course Support Form and include the Influenza Outbreak Investigation PCR forms already completed for you for this kit, but please double check the quantities requested for the number of students you have using the information above. These forms include:
 - a. Educational Course Support Form (add your contact information)
 - b. Annotated protocol showing how much of each reagent is used per student
 - c. NEB Summary Table (Excel Sheet)
 - d. Quantity Order Sheet