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ProductInformation

QuickCombä -96
Porous Membrane Loading and Storage Comb

Product No. **C 3226**
Store at room temperature

Product Description

Sigma's QuickComb™-96 provides a sturdy, reliable membrane comb that allows for easy bench-top sample loading and storage in one convenient product. Simply spot samples on the individual teeth, insert the comb, and electrophorese. During electrophoresis, the entire sample is drawn from the porous teeth resulting in maximum signal strength. With no wells to load, lane-to-lane leeching is eliminated and lane tracking becomes much easier.

Precautions and Disclaimer

Sigma's QuickComb-96 is for laboratory use only. Not for drug, household or other use.
Always wear gloves when handling QuickCombs to prevent any residual dirt and oil on fingers from contaminating the surface of the comb. Handle the QuickComb carefully to prevent inadvertent bending or tearing of teeth.

Equipment Required But Not Provided

- Single or Multichannel pipette that dispenses 0.1–10 µl or less (0.5 µl maximum load volume for teeth of comb).

Storage

- Unused QuickCombs can be stored in their original packaging at room temperature indefinitely.
- Spotted combs can be stored at 4 °C for up to 2 weeks or –20 °C for 3-6 months.
- Spotted combs should also be kept in the dark in a protective container for any cold storage.

Procedure

A. Loading Sample onto the QuickComb

1. While wearing gloves, remove a QuickComb from its original protective sleeve and place on clean and dry benchtop surface for loading. Be sure that the non-laminated side of each QuickComb tooth is lying face up.

2. Reaction samples should be prepared for loading either by standard protocols or by comparable means. Sigma recommends resuspending each dried sequencing product in 2 µl of loading buffer (PE Biosystems: 25 mM EDTA/ blue dextran, 50 mg/ml deionized formamide), followed by heat denaturation for 2 minutes at 94 °C.

3. After denaturation, draw up 0.2-0.5 µl of sample in a single or multi-channel pipette.

4. Carefully “spot load” each sample onto the non-laminated side of a designated individual tooth of the comb. It is important to release the samples from the pipette slightly above the membrane such that the droplet makes contact with the membrane allowing for sample absorption. The entire sample can be drawn onto the tooth in about 2–5 seconds.

5. After all samples have been loaded, wait approximately 30-60 seconds to ensure that the entire sample has been absorbed into the porous material.

   Note: At this time the QuickComb can either be inserted for electrophoresis as indicated below or stored in the dark at 4 °C or –20 °C.

B. Inserting Comb and Starting the Run Gel

1. If the QuickComb has been spotted and stored at 4 °C or at –20 °C, do not remove until ready for insertion into the gel.

2. Prepare the gel for loading according to the manufacturer's protocol.

   a. Remove the casting comb and clean the sample loading region thoroughly to ensure the area is free of gel debris.

      Note: Some labs use binder clips to secure the casting comb between the plates of freshly poured gels. When pouring gels that are to be loaded using a QuickComb it is important **NOT** to use binder clips to secure the casting comb. Uneven pressure on the glass plates caused by using binder clips can make it extremely difficult to insert the QuickComb for loading.

   b. Mount the gel onto the gel cassette and thoroughly clean the laser read area using standard laboratory protocols. Individuals should use a residue-free wiping material (e.g. Kimwipes) and deionized water and/or isopropanol to ensure the region is free of any excess acrylamide or dirt.

   c. Place the gel cassette onto the sequencing instrument and initiate a plate check according to the manufacturer's instructions.

   d. Once a satisfactory plate check is observed, fasten the upper buffer chamber and add 594 ml of **deionized water**. Then add 660 ml of 1X TBE running buffer to the lower buffer chamber.

   e. Initiate the desired pre-run module (e.g. 36E–1200) and immediately pause the instrument. This allows the pre-run to continue without initiating electrophoresis until the gel temperature reaches 51 °C (approximately 25-35 minutes). Use this time to ensure that the required sample sheet is complete and ready for use with the corresponding run.

3. After the gel has reached temperature, cancel the pre-run. Start the data collection program and immediately pause the instrument

   Note: It is very important to be able to quickly initiate the run after the QuickComb has been inserted. If not, the samples will diffuse out of the QuickComb into the buffer chamber, resulting in lane-to-lane leaking.

4. Once the collection has been initiated and the run paused, open the instrument door and thoroughly rinse out the loading region with a 60 cc syringe filled with water from the upper chamber.

5. Quickly and carefully insert the pre-loaded QuickComb between the front and back plate of the gel in the upper buffer chamber. It can be helpful to anchor the teeth against the back plate, slightly bend the top of the comb, and gently slide the QuickComb into the gel. This technique helps to reduce surface tension created when the body of the comb meets the wet surface of the back plate. It is important that the teeth of the comb meet flush with the well face of the gel created by the casting comb.

6. After the QuickComb has been inserted, close the instrument door and resume the run for 30-45 seconds. At this point the entire sample is being electrophoresed into the gel.

7. Pause the instrument again, open the door, and remove the QuickComb.

8. **To change the water in the upper buffer chamber to a 1X buffer solution, add 66 ml of 10X TBE and gently swirl to mix**.

9. Once the buffer is carefully mixed, close the door and resume the run.

Troubleshooting Guide

Problem	Cause	Solution
Difficulty inserting the QuickComb-96 between the glass plates of the gel	Use of binder clips to secure the casting comb between the plates of freshly poured gels. Uneven pressure on the glass plates in this area can make it extremely difficult to insert the QuickComb for loading.	When pouring gels that are to be loaded using a QuickComb, it is important to avoid doing this. Excess material may be cut from the ends of the QuickComb-96 to ease insertion between notches of front glass plate.
Faint signal for QuickComb samples that are being electro-phoresed for a second time	The QuickComb-96 is a single use product. Nearly the entire sample is drawn out of the porous material during electrophoresis.	Use each QuickComb only once. If you do not have enough samples to load a full QuickComb, cut the comb to the size needed.
Gel Image looks fine, but electropherograms show poor and incorrect basecalling	Electrophoresis of samples from the QuickComb into the gel using 1X TBE buffer in the upper chamber will cause co-migration of fragments that cannot be resolved by the ABI basecaller.	Electrophorese samples into the gel with deionized water in the upper buffer chamber only.
Samples seem to leak from lane-to-lane, causing a fuzzy image	Electrophoresis of samples from the QuickComb into the gel using 1X TBE buffer in the upper chamber will cause co-migration of fragments that cannot be resolved by the ABI basecaller.	Set up the instrument run so electro-phoresis can be initiated immediately after the QuickComb is inserted. This will help create a more defined sample lane with better lane to lane spacing. The amount of time between insertion of the QuickComb and electrophoresis should be reduced as much as possible for optimal results.

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