Steps to Diluting Methacholine Chloride
US Package Insert - Single Patient

Getting Started

1. Attach labels to sterile empty vials.
2. Wipe down the stoppers of the Provocholine vial, diluent vial, and sterile empty vials with alcohol prep pads.
3. Label two (2) appropriately sized syringes (one for Provocholine, one for diluent), and attach needles to each.

Preparing Vial A
25 mg/mL Solution

1. Using the diluent needle and syringe, draw 4 mL of diluent and transfer the 4 mL of diluent to the Provocholine 100 mg vial.
2. Shake well.

Preparing Vial B
10 mg/mL Solution

1. Using the Provocholine needle and syringe, draw 1 mL of solution from Vial A and transfer to Vial B.
2. Using the diluent needle and syringe, draw 1.5 mL of diluent and transfer to Vial B.
3. Shake well.

Preparing Vial C
2.5 mg/mL Solution

1. Using the Provocholine needle and syringe, draw 1 mL of solution from Vial A and transfer to Vial C.
2. Using the diluent needle and syringe, draw 9 mL of diluent and transfer to Vial C.
3. Shake well.

NOTE
1. Vial E must be prepared on the day of the challenge test.
2. Transfer all dilutions (in vials A through E) to nebulizer through the 0.22 µm sterile bacterial retentive filter (Millex GV®)*
3. Be sure to follow your institutional policy for needle and sharps safety.

*Do not attach filter until AFTER solution has been drawn into syringe.
For technical support, please contact us Toll-Free at 1-833-887-7686

**Preparation of Solutions**

**Preparation of Vial D**

1. Using the Provoccholine needle and syringe, draw 1 mL of solution from Vial C and transfer to Vial D.
2. Using the diluent needle and syringe, draw 9 mL of diluent and transfer to Vial D.
3. Shake well.

**Preparation of Vial E**

1. Using the Provoccholine needle and syringe, draw 1 mL of solution from Vial D and transfer to Vial E.
2. Using the diluent needle and syringe, draw 9 mL of diluent and transfer to Vial E.
3. Shake well.

**Dilution Check Sheet and Control Record**

**PROVOCHELINE DILUTIONS FOR CHALLENGE TESTING**

<table>
<thead>
<tr>
<th>Take Provoccholine</th>
<th>Add diluent (Shake well!)</th>
<th>Obtain dilution</th>
<th>Vial name</th>
<th>Initial / Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provoccholine 100 mg</td>
<td>4 mL</td>
<td>25 mg/mL</td>
<td>Vial A - 25 mg/mL</td>
<td></td>
</tr>
<tr>
<td>1 mL from Vial A</td>
<td>1.5 mL</td>
<td>10 mg/mL</td>
<td>Vial B - 10 mg/mL</td>
<td></td>
</tr>
<tr>
<td>1 mL from Vial A</td>
<td>9 mL</td>
<td>2.5 mg/mL</td>
<td>Vial C - 2.5 mg/mL</td>
<td></td>
</tr>
<tr>
<td>1 mL from Vial C</td>
<td>9 mL</td>
<td>0.25 mg/mL</td>
<td>Vial D - 0.25 mg/mL</td>
<td></td>
</tr>
<tr>
<td>1 mL from Vial D</td>
<td>9 mL</td>
<td>0.025 mg/mL</td>
<td>Vial E - 0.025 mg/mL</td>
<td></td>
</tr>
</tbody>
</table>

**Storage Instructions**

- Provoccholine powder should be stored at 59°F to 86°F (15°C to 30°C).
- Dilutions A through D (25 mg/mL through 0.25 mg/mL) should be stored at 36°F to 46°F (2°C to 8°C) in a refrigerator for no more than 2 weeks.
- Vial E (0.025 mg/mL) must be prepared on the day of the challenge test.
- Be sure to remove the Provoccholine dilutions from the refrigerator 30 minutes prior to testing to allow them to warm to room temperature (59°F to 77°F or 15°C to 25°C as per World Health Organization).

**For technical support, please contact us Toll-Free at 1-833-887-7686**