MICE: CHOOSE 1 STRAIN

DBA/1 (DBA) mice OR C57BL/6 (B6) mice

- 7-8 weeks old
- SPF housing conditions
- High fat diet

RECOMMENDED ADJUVANTS & COLLAGENS: CHOOSE 1 ADJUVANT + 1 COLLAGEN

<table>
<thead>
<tr>
<th>Catalog #</th>
<th>Adjuvants</th>
<th>Catalog #</th>
<th>Collagens</th>
</tr>
</thead>
<tbody>
<tr>
<td>7002</td>
<td>Incomplete Freund’s Adjuvant (IFA) (DBA - Booster only)</td>
<td>20011</td>
<td>Chick Type II Collagen, Lyophilized (DBA or B6)</td>
</tr>
<tr>
<td>7008</td>
<td>Complete Freund’s Adjuvant (CFA), 1 mg/ml (DBA)</td>
<td>20012</td>
<td>Chick Type II Collagen, 2 mg/ml Solution (DBA or B6)</td>
</tr>
<tr>
<td>7001</td>
<td>Complete Freund’s Adjuvant (CFA), 4 mg/ml (DBA)</td>
<td>20021</td>
<td>Bovine Type II Collagen, Lyophilized (DBA)</td>
</tr>
<tr>
<td>7023</td>
<td>Complete Freund’s Adjuvant (CFA), 5 mg/ml (B6)</td>
<td>20022</td>
<td>Bovine Type II Collagen, 2 mg/ml Solution (DBA)</td>
</tr>
</tbody>
</table>

IMMUNIZATION SCHEDULE: CHOOSE 1 OPTION

OPTION 1: Single Immunization Protocol (DBA mice)

Day 0: Subcutaneous injection: Collagen emulsion with CFA: 4 mg/ml *M. tuberculosis*

Day 0 - 28: Score arthritis every 7 days

Day 28 - 56: Score arthritis every 2-3 days: Arthritis incidence 90 - 100%

Day 56: End of experiment.

OR

OPTION 2: Booster Immunization Protocol

Day 0: Subcutaneous injection: Collagen emulsion with CFA: 1 mg/ml (DBA) or 5 mg/ml *M. tuberculosis* (B6)

Day 0 - 28: Score arthritis every 7 days

Day 21: Subcutaneous injection (booster): Collagen emulsion with IFA (DBA) or CFA: 5 mg/ml *M. tuberculosis* (B6)

Day 28 - 56: Score arthritis every 2-3 days: Arthritis incidence 90 - 100% (DBA), 50-70% (B6)

Day 56: End of experiment
PREPARE EMULSION WITH A HOMOGENIZER: DO NOT USE THE DOUBLE-SYRINGE METHOD!

Procedure*:

1. Gather materials (Figure 1. Homogenizer blade (a), 10 ml syringe with a 3-way stopcock (b), and 1 ml Hamilton syringe (c)).
2. Lock stopcock.
3. Keep setup on ice (Figure 2).
4. Add CFA to syringe.
5. Add an equal volume of collagen solution, drop-wise (Figure 3).
6. Mix at 10,000 - 30,000 rpm for 2 minutes. Move the syringe up and down while mixing.
7. Cool syringe on ice for 5 minutes.
8. Mix at 10,000 - 30,000 rpm for 2 minutes. Move the syringe up and down while mixing.
9. Evaluate emulsion with the drop test (next page).
10. Transfer the emulsion to a Hamilton syringe using the 3-way stopcock.

*For more information, please see Chondrex, Inc’s Mouse CIA Protocol
EMULSION DROP TEST

Emulsion in 10 ml syringe

Cold H₂O

- Emulsion holds shape: Ready to use!
  (Use immediately or store at 4°C up to 3 hours)
- Emulsion disperses on water surface
  (Add one drop of adjuvant to the emulsion and homogenize again)

IMMUNIZATION

(a) Emulsion in Hamilton Syringe
   (no air bubbles!)
(b) Needle (25 or 27-gauge x 5/8”)
(c) Restraining Device
**SUBCUTANEOUS INJECTION:** 0.1 ml per injection

**AFTER INJECTION:**
- DO NOT USE IP (a) OR IV (b) INJECTIONS TO IMMUNIZE MICE!
- See emulsion band under skin

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