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

- **See emulsion band under skin**

**DO NOT USE**

IP (a) OR IV (b)
INJECTIONS TO
IMMUNIZE MICE!