Mouse CIA – A Visual Guide

For Research Use Only - Not Human or Therapeutic Use

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

Catalog #	Adjuvants
7002	Incomplete Freund's Adjuvant (IFA) (DBA - Booster only)
7008	Complete Freund's Adjuvant (CFA), 1 mg/ml (DBA)
7001	Complete Freund's Adjuvant (CFA), 4 mg/ml (DBA)
7023	Complete Freund's Adjuvant (CFA), 5 mg/ml (B6)

Catalog #	Collagens*
20011	Chick Type II Collagen, Lyophilized (DBA or B6)
20012	Chick Type II Collagen, 2 mg/ml Solution (DBA or B6)
20021	Bovine Type II Collagen, Lyophilized (DBA)
20022	Bovine Type II Collagen, 2 mg/ml Solution (DBA)

*Chondrex, Inc. also offers Cat # 9075 Collagen Solubilizing Buffer (0.05M Acetic Acid) for dissolving or diluting collagen

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

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PREPARE EMULSION WITH A HOMOGENIZER: DO NOT USE THE DOUBLE-SYRINGE METHOD!

Figure 1

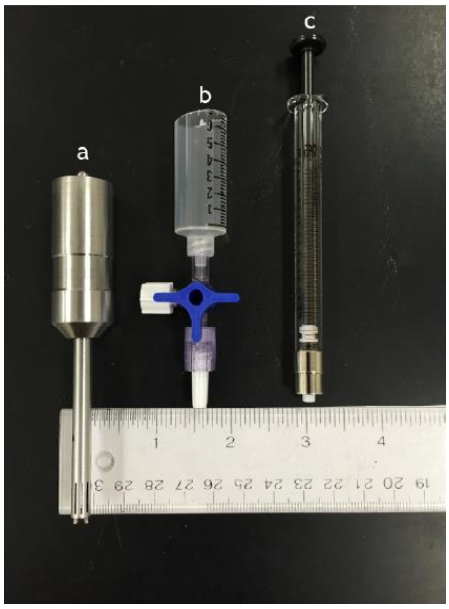
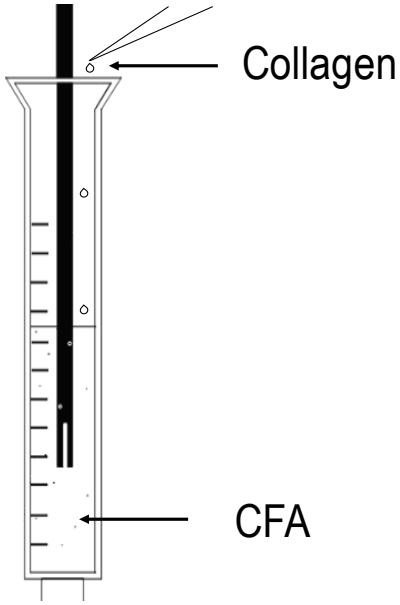


Figure 2

Figure 3



Procedure*:

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

*For more information, please see Chondrex, Inc's **Mouse CIA Protocol**

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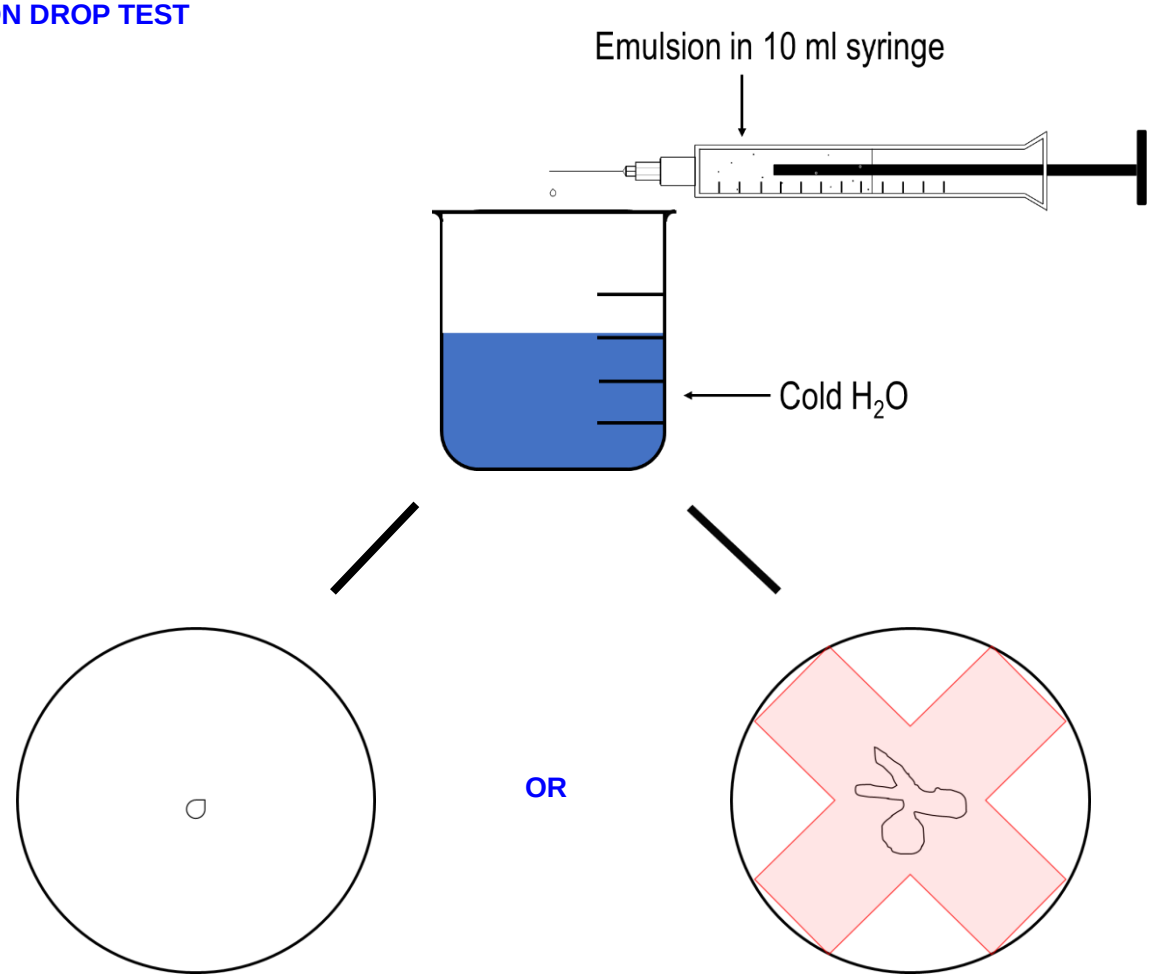
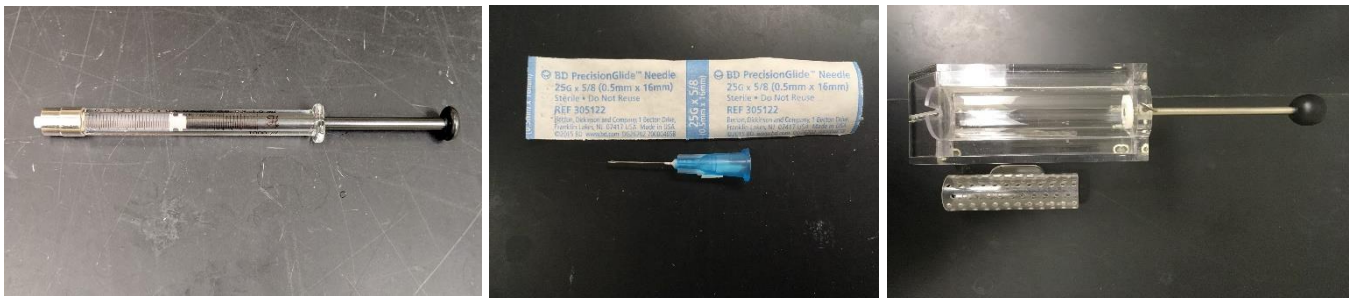
EMULSION DROP TEST



Emulsion holds shape: Ready to use!
(Use immediately or store at 4ºC 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

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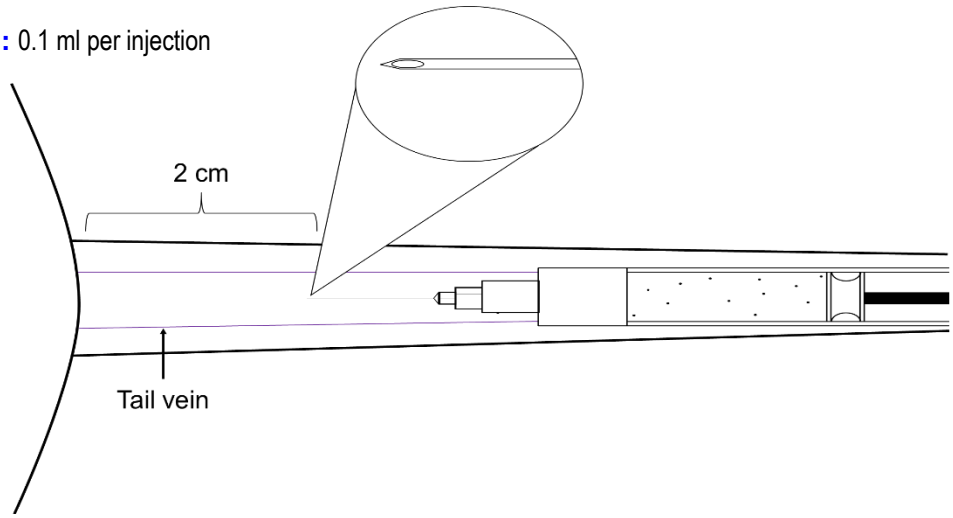
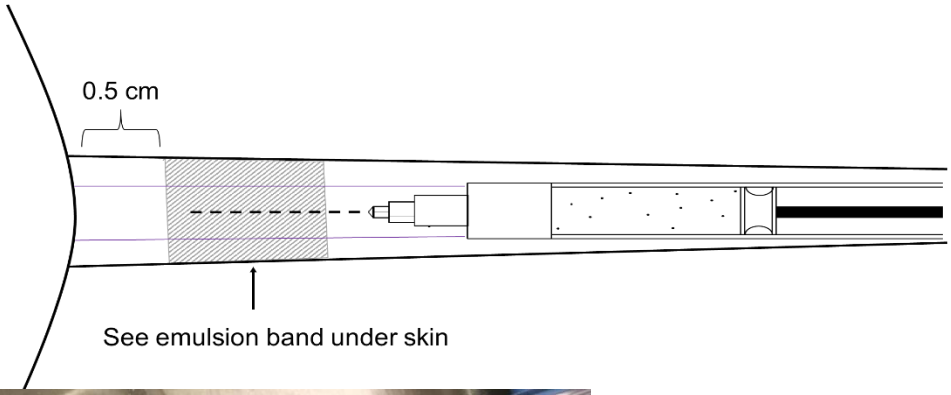
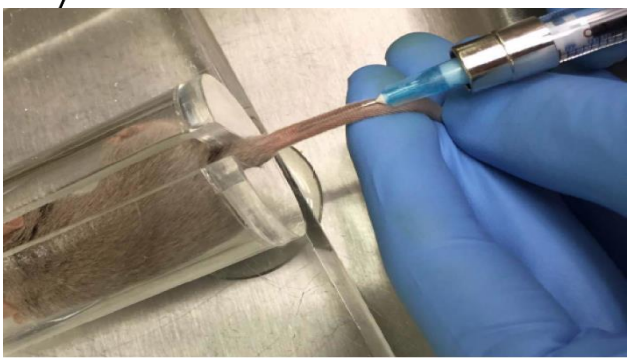
SUBCUTANEOUS INJECTION: 0.1 ml per injection



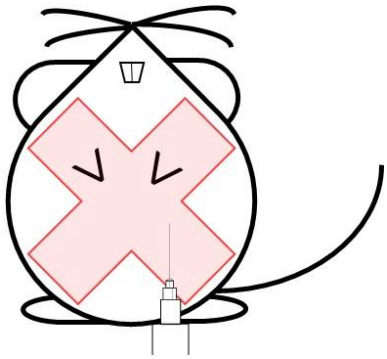
AFTER INJECTION:

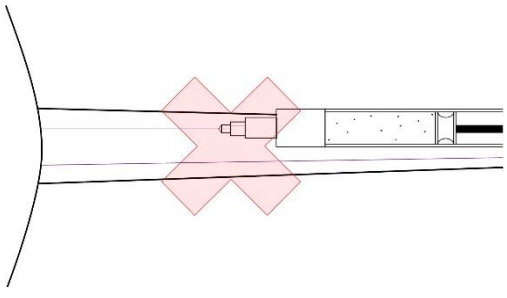




DO NOT USE IP (a) OR IV (b) INJECTIONS TO IMMUNIZE MICE!

(a)





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