A Protocol for Successfully Inducing Collagen-Induced Arthritis (CIA) in C57BL/6 Mice

For Research Use Only - Not Human or Therapeutic Use

BACKGROUND

Collagen-induced arthritis (CIA) in mice shares immunological and pathological features with human rheumatoid arthritis (RA). The CIA model is ideal for studying the pathogenesis of RA and to evaluate potential therapeutics (1-3). Although the model is highly reproducible, several factors must be carefully considered to achieve arthritis with sufficient incidence and severity. Therefore, a pilot study is strongly recommended for first time users of this animal model, especially for inducing CIA in low responding C57BL/6 mice.

Alternatively, collagen antibody-induced arthritis (CAIA) from Chondrex, Inc. can reliably induce arthritis at 100% incidence and high severity at a dose of 5 mg/mouse in C57BL/6 mice. For more information, please visit our website www.chondrex.com or contact the support team at support@chondrex.com.

A. Animal Vendors

From vendor to vendor and even within the same strain, the genetic background and bacteria flora will vary among mice. These differences affect how the mice will respond to various reagents, thus impacting experimental results (4). Chondrex, Inc. recommends testing animals from different vendors using a defined protocol before proceeding with a full-scale experiment.

B. Housing Condition & Diet

Chondrex, Inc. recommends housing animals under Specific Pathogen Free (SPF) conditions rather than conventional conditions to minimize variations within experiments caused by bacterial and viral infections. For example, mice infected with mouse hepatitis virus (MHV) will not develop CIA (unpublished observation). Additionally, the incidence and severity of arthritis can differ among mice fed different commercially available rodent chows. The highest incidence of disease has been observed in mice that were fed a high-fat diet designed for breeders. (LabDiet Mouse Chow 5015) (5).

C. Mouse Age & Strains

Mice should be at least 7-8 weeks old with a mature immune system. Aged mice may exhibit lower incidence and severity. Chondrex, Inc. recommends consistently using the same age for better reproducibility in repeat studies. Susceptibility to CIA is linked to MHC-class II molecules which respond to individual species of type II collagen used for immunization (6). Although C57BL/6 (H-2^b) mice respond poorly to mouse, bovine, and porcine type II collagen, chick type II collagen, under a specific immunization protocol can induce autoantibodies cross-reacting with mouse type II collagen, resulting in successful induction of arthritis.

A list of mouse strains commonly used for CIA and CAIA are shown in Table 1.

Table 1 - Mouse strains commonly used for CIA and CAIA

Mouse Strain	H-2 Type	CIA Susceptibility	Ref #	CAIA Susceptibility	Ref #	Note
DBA/1	q	High	2, 5, 6	High	9, 11	INFγ high
B10.RIII	r	High	6	High	9	Low response: chick and human type II
B10	b	Low	8	(High)		* Need alternative immunization
C57BL/6	b	Low	8	Moderate - High	7,10,12	LPS low responder –
Balb/c	d	Resistant		High	9	
C3H/He	k	Low	13	(Low)		
SJL/1	s	Moderate	2	(High)		

D. Adjuvant

Complete Freund's Adjuvant (CFA), consisting of high-quality *M. tuberculosis*, is essential to induce severe arthritis in mice because it induces a strong immune response. CFA containing 5 mg/ml of *M. tuberculosis* (Cat # 7023) successfully induced arthritis with sufficient incidence (50-70%) in CIA low-responding mouse strains, such as C57BL/6 (H-2^b) (8). However, because high concentrations of *M. tuberculosis* induce severe inflammation at the injection sites, please contact your institution's animal committee for approval of use of the CFA. **Chondrex, Inc. recommends the following adjuvant for inducing CIA in C57BL/6 mice**.

Catalog #	Description
7023	Complete Freund's Adjuvant, 5 ml x 5 mg/ml

E. Collagen

Native, highly purified type II collagen prepared under a defined protocol should be used as deglycosylation of collagen will affect the arthritogenicity (14). Moreover, the failure to remove minor contaminants such as pepsin likely yields false positive reactions in T-cell proliferation assays (15). Lyophilized collagen is very stable if properly stored at -20°C in the dark. Collagen should be dissolved at 2-4 mg/ml in 0.05M acetic acid (Cat # 9075) by gently stirring overnight at 4°C. Collagen solutions can be kept at 4°C for one week but should then be kept at -20°C thereafter.

© 2026 Chondrex, Inc. All Rights Reserved, MCIA C57BL/6 1.0

Chondrex, Inc. recommends immunization grade chick type II collagen for the CIA model in C57BL/6 mice.

Catalog #	Description
20011	Chick type II collagen, 10 mg
20012	Chick type II collagen, 5 ml x 2 mg/ml
9075	Collagen Solubilizing Buffer (0.05M Acetic Acid), 5 ml

PROTOCOL TO INDUCE CIA

Chondrex, Inc. provides a visual guide to induce CIA

A. Preparing the Emulsion

A high-quality emulsion for immunization is critical for inducing arthritis with high incidence, especially with C57BL/6 mice. Emulsions can be made using various methods. However, double-syringe or sonication methods are not recommended. These methods yield emulsions that are not stable enough to effectively induce arthritis. In addition, sonication cleaves collagen into fragments which will be denatured at body temperature.

An electric homogenizer is highly recommended for preparing an emulsion:

VIDEO: Emulsion Preparation

1. Use a homogenizer (Figure 1) with a small blade (diameters of 5 mm or less) to emulsify the CFA with the collagen solution (Figure 2a). Seal the tip of the syringe with a 3-way stopcock Figure 2b). Next, clamp the syringe to a ring stand and place it in an ice water bath to keep the emulsion cool during mixing, as heat will denature the collagen which will then fail to induce arthritis (Figure 3).

Figure 1 – Homogenizer (Ex: OMNI, TH115)

Figure 2 - Homogenizing blade - 0.5 cm diameter (a), Syringe with a 3-way stopcock (b), Hamilton glass syringe - 1 ml (c).

Figure 3 - A syringe sealed with a 3-way stopcock, clamped to a stand, and placed in an ice water bath.

2. Add one volume (maximum: 25% of the syringe volume) of CFA to the end of the syringe sealed with the 3-way stopcock. Then gradually add an equal volume of collagen solution (2 mg/ml in 0.05M acetic acid) dropwise while mixing at low speed (1000-3000 rpm).

NOTE: To ensure a high-quality emulsion, the maximum emulsion volume should be no more than half of the syringe volume (50%). If more is needed, make several batches.

3. Continue mixing the emulsion at maximum speed (approximately 10,000 - 30,000 rpm) for 2 minutes. Cool down the emulsion by keeping the syringe in the ice water bath for 5

© 2026 Chondrex, Inc. All Rights Reserved, MCIA C57BL/6 1.0

minutes. Repeat mixing and cooling 2-3 times. For larger volumes (2-5 ml), we suggest moving the blade throughout the emulsion while mixing for better uniformity.

4. Replace the stopcock with a needle and test the stability of the emulsion by adding one drop of emulsion into a beaker of water. If the emulsion is stable, the drop will remain as a solid clump which does not dissipate on the water's surface (Figure 4). If the emulsion dissipates onto the water surface, then the emulsion is not stable. Add a few drops of adjuvant, mix again, and retest.

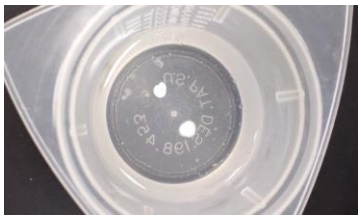

Figure 4 - An intact, stable emulsion on the water's surface

5. Transfer the emulsion to a 1 ml Hamilton glass syringe (Figure 2c). Injecting an accurate volume of emulsion is difficult with a plastic syringe.

NOTE 1: Remove air bubbles from the emulsion by forcefully swinging your arm towards the floor, with the Hamilton syringe in hand (plunger side down).

NOTE 2: Chondrex, Inc. recommends injecting the collagen emulsion within a couple of hours from preparation. Keep the emulsion at 4°C until use

B. Injection Site

Place a 25 or 27-gauge x 5/8" needle on the Hamilton syringe. Before each injection, wipe the needle to prevent leakage of the emulsion. Insert the needle bevel side up and parallel to the tail 2 cm from the base of the tail until the needle tip is 0.5 cm from the base. The entire needle should be subcutaneous. Inject 0.1 ml (100 µg collagen/mouse) of the emulsion subcutaneously at the base of the tail (Figure 5). For a booster injection, insert the needle at 3 cm from the base of the tail until the tip reaches 1.5 cm from the base. The booster injection should be administered at a different location from the initial injection.

VIDEO: SC Injection

NOTE: Chondrex, Inc. does not recommend subcutaneous injections in the back nor intraperitoneal (IP) injections, as emulsions cause severe inflammatory reactions in the peritoneal and thoracic cavities.

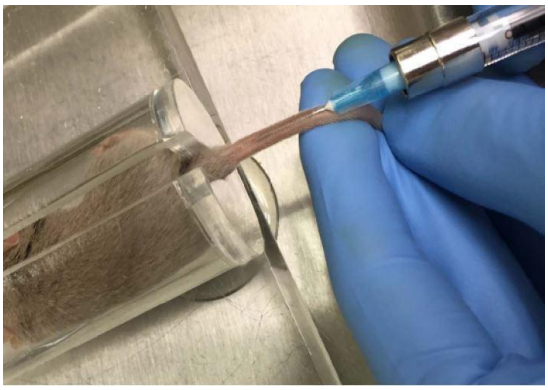

Figure 5 - Subcutaneous Immunization of Emulsion.

C. Immunization Schedule for C57BL/6 mice

Inject the collagen and CFA emulsion containing a final concentration of 2.5 mg/ml of *M. tuberculosis*. Administer a booster injection with an emulsion of collagen and CFA containing a final concentration of 2.5 mg/ml of *M. tuberculosis* on day 21. As an example, arthritis will develop on days 28-35 after the first immunization. The maximum incidence of arthritis in these mice reaches approximately 50-70% on days 42-56 (10). However, the specific timeline is highly dependent on animal vendors and housing conditions.

Optional Protocol: Synchronizing Onset and Increasing Incidence via LPS Injection

LPS (a B-cell mitogen) exerts a synergistic effect in triggering arthritis with sub-arthritogenic doses of autoantibodies to type II collagen, resulting in significantly higher severity and incidence in the CIA (9,16-18). The bacterial toxins can be used not only to trigger and enhance arthritis, but also to synchronize the onset of arthritis.

To implement this modification, follow the primary immunization protocol and inject LPS (25-50 µg in saline) intraperitoneally on day 25-28 or 3-5 days before the desired onset of arthritis. Following the administration, arthritis onset is typically observed within 24–48 hours, with a high incidence rate in the mice.

NOTE: Mice immunized with CFA develop severe immune suppression for 2-4 weeks following the first immunization. Therefore, some mice will be highly susceptible to LPS injection (50 µg). As previously mentioned, (see Animal Vendors), Chondrex, Inc. suggests testing animals from different vendors before proceeding with a full-scale experiment.

Catalog #	Description
9028	Lipopolysaccharide (LPS), O111:B4

© 2026 Chondrex, Inc. All Rights Reserved, MCIA C57BL/6 1.0

EVALUATING ARTHRITIS

A. Scoring

Disease can be assessed by a qualitative clinical score or by determining paw thickness using a thickness gauge, such as a Mitutoyo loop handle dial thickness gauge with a round disc. These methods are applicable for all arthritis models including CIA, adjuvant-induced arthritis, CAIA, and other inflammatory models. Chondrex, Inc. provides a scoring system (Table 2) and a supplemental flyer.

NOTE: Mouse paw volume cannot be determined by a plethysmograph as used for rat paw volume measurement because the mouse paw is too small.

Table 2 - Qualitative scoring system used to assess severity of paw inflammation.

Score	Condition
0	Normal
1	Mild, but definite redness and swelling of the ankle or wrist, or apparent redness and swelling limited to individual digits, regardless of the number of affected digits
2	Moderate redness and swelling of ankle or wrist
3	Severe redness and swelling of the entire paw including digits
4	Maximally inflamed limb with involvement of multiple joints

B. Serum Analysis

High IgG autoantibody levels to mouse type II collagen are important for inducing arthritis (8, 19). **More specifically, high levels of anti-type II collagen IgG2b and IgG2c subtype antibodies in C57BL/6 mice are required to activate complement, an essential step for inducing arthritis; it is important to note that C57BL/6 mice do not produce IgG2a subtype antibodies** Chondrex, Inc. provides mouse Anti-Collagen antibody ELISA kits to analyze the antibody levels. Please visit the product pages for anti-Collagen IgG and IgG subtype for more information.

REFERENCES

1. D. Trentham, A. Townes, A. Kang, Autoimmunity to Type II Collagen an Experimental Model of Arthritis. *J Exp Med* **146**, 857-68 (1977).

2. J. Courtenay, M. Dallman, A. Dayan, A. Martin, B. Mosedale, Immunisation Against Heterologous Type II Collagen Induces Arthritis in Mice. *Nature* **283**, 666-8 (1980).

3. E. Cathcart, K. Hayes, W. Gonnerman, A. Lazzari, C. Franzblau, Experimental Arthritis in a Nonhuman Primate. I. Induction by Bovine Type II Collagen. *Lab Invest* **54**, 26-31 (1986).

4. Ivanov, K. Atarashi, N. Manel, E. Brodie, T. Shima, *et al*., Induction of Intestinal Th17 *Cell*s by Segmented Filamentous Bacteria. Cell **139**, 485-98 (2009).

5. P. Wooley, Collagen-induced arthritis in the mouse. Methods Enzymol 162:361-373, 1988.

6. P. Wooley, H. Luthra, M. Griffiths, J. Stuart, A. Huse, C. David, *et al*., Type II Collagen-Induced Arthritis in Mice. IV. Variations in Immunogenetic Regulation Provide Evidence for Multiple Arthritogenic Epitopes on the Collagen Molecule. *J Immunol* **135**, 2443-51 (1985).

7. W. Watson, A. Townes, Genetic Susceptibility to Murine Collagen II Autoimmune Arthritis. Proposed Relationship to the IgG2 Autoantibody Subclass Response, Complement C5, Major Histocompatibility Complex (MHC) and non-MHC Loci. *J Exp Med* **162**, 1878-91 (1985).

8. I. Campbell, J. Hamilton, I. Wicks, Collagen-induced Arthritis in C57BL/6 (H-2b) Mice: New Insights Into an Important Disease Model of Rheumatoid Arthritis. *Eur J Immunol* **30**, 1568-75 (2000).

9. K. Terato, D. Harper, M. Griffiths, D. Hasty, X. Ye, *et al*., Collagen-induced Arthritis in Mice: Synergistic Effect of E. Coli Lipopolysaccharide Bypasses Epitope Specificity in the Induction of Arthritis With Monoclonal Antibodies to Type II Collagen. *Autoimmunity* **22**, 137-47 (1995).

10. T. Kagari, H. Doi, T. Shimozato, The Importance of IL-1 Beta and TNF-alpha, and the Noninvolvement of IL-6, in the Development of Monoclonal Antibody-Induced Arthritis. *J Immunol* **169**, 1459-66 (2002).

11. K. Terato, K. Hasty, R. Reife, M. Cremer, A. Kang, J. Stuart, *et al*., Induction of Arthritis With Monoclonal Antibodies to Collagen. *J Immunol* **148**, 2103-8 (1992).

© 2026 Chondrex, Inc. All Rights Reserved, MCIA C57BL/6 1.0

12. H. Kato, K. Nishida, A. Yoshida, I. Takada, C. McCown, *et al*., Effect of NOS2 Gene Deficiency on the Development of Autoantibody Mediated Arthritis and Subsequent Articular Cartilage Degeneration. *J Rheumatol* **30**, 247-55 (2003).

13. R. Holmdahl, L. Jansson, M. Andersson, E. Larsson, Immunogenetics of Type II Collagen Autoimmunity and Susceptibility to Collagen Arthritis. *Immunology* **65**, 305-10 (1988).

14. E. Michaëlsson, V. Malmström, S. Reis, A. Engström, H. Burkhardt, R. Holmdahl, *et al*., T Cell Recognition of Carbohydrates on Type II Collagen. *J Exp Med* **180**, 745-9 (1994).

15. M. Andersson, R. Holmdahl, Analysis of Type II Collagen-Reactive T Cells in the Mouse. I. Different Regulation of Autoreactive vs. Non-Autoreactive Anti-Type II Collagen T Cells in the DBA/1 Mouse. *Eur J Immunol* **20**, 1061-6 (1990).

16. S. Yoshino, E. Sasatomi, Y. Mori, M. Sagai, Oral Administration of Lipopolysaccharide Exacerbates Collagen-Induced Arthritis in Mice. *J Immunol* **163**, 3417-22 (1999).

17. B. Cole, M. Griffiths, Triggering and Exacerbation of Autoimmune Arthritis by the Mycoplasma Arthritidis Superantigen MAM. *Arthritis Rheum* **36**, 994-1002 (1993).

18. Y. Takaoka, H. Nagai, M. Tanahashi, K. Kawada, Cyclosporin A and FK-506 Inhibit Development of Superantigen-Potentiated Collagen-Induced Arthritis in Mice. *Gen Pharmacol* **30**, 777-82 (1998).

19. R. Reife, N. Loutis, W. Watson, K. Hasty, J. Stuart, SWR Mice Are Resistant to Collagen-Induced Arthritis but Produce Potentially Arthritogenic Antibodies. *Arthritis Rheum* **34**, 776-81 (1991).

© 2026 Chondrex, Inc. All Rights Reserved, MCIA C57BL/6 1.0

16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com