

Product Name

Collagen Antibody Induced Arthritis (CAIA) in mice

CAT No.

MQ 18.101

LOT No.

15238-7,14mg

Quantity

50 mg

Edition: February 1, 2012

Intended use

This product is for research use only. NOT for use in diagnostic or therapeutic procedures.

Mouse monoclonal antibodies to mouse collagen type II. Intended for use in the CAIA mouse model.

Reagent provided

Mixture of 8 antibodies that recognize 7 different collagen type II epitopes, as tested in a competition assay.

Isotypes

IgG1, IgG2a, IgG2b

Immunogen

Bovine collagen type II, isolated from the joint.

Specificity

Specificity has been tested in ELISA. In Figure 1 anti-type II collagen clone RC7 is depicted which is representative for all 8 clones in the antibody mixture. Cross reactivity with mouse type II collagen (Chondrex) has been detected. Additional tests for cross reactivity have not yet been performed.

Purity

Protein A purified.

Precautions

1. For professional users.
2. As with any product derived from biological sources, proper handling procedures should be used.

Preparation of the antibody

The provided antibody mix is lyophilized and needs to be reconstituted in PBS (20 mg/ml).

Recommended concentration: 2.4-3.2 mg/mouse.

Storage instructions

Antibodies are lyophilized and shipped at room temperature. Upon arrival store at -20°C. After reconstitution in PBS (20 mg/ml), use directly or store at -20°C. If you need to store for a short time, avoid freeze thawing and store at 4°C.

Relevance

Collagen-induced arthritis is an antibody mediated disease. Therefore, type II collagen specific antibodies can be used to induce arthritis. Our mixture of type II collagen specific antibodies (2.4 mg per animal) induces severe inflammation in the ankle and paws of DBA mice (fig. 2 and 4) which resemble many aspects of arthritis. In table 1 an explanation is given of the different arthritis scores. A dose titration of the antibodies showed that the lowest dose (0.8 mg) showed arthritis in 33.3% of the mice which increased to 83.3% after LPS injection (Fig. 3). One of the biggest advantages of our monoclonal antibody-induced (mAb) arthritis model is that severe arthritis is induced within 24-48 hours instead of the four weeks required to induce arthritis after immunization with type II collagen. ModiQuest Research recommends highly

susceptible DBA/1 mice. Other strains may be used, however the amount of antibody cocktail may vary. The current MQR-CAIA model is ideal for rapidly screening therapeutics for the treatment of arthritis and to study the mechanisms involved in the development of arthritis.

Procedure

- 1) On day 0 administer mAb cocktail intra-peritoneal (i.p.) diluted in 0.9% Natriumchloride in between 2.4 mg and 3.2 mg per animal.
- 2) Dissolve LPS in 0.9% Natriumchloride by thorough vortexing and administer 25 ug/animal i.p. In-between day 3 and 6 if desirable.
- 3) Observe arthritis score throughout the study on a daily basis. Initial symptoms of arthritis typically appear on day 2.

Mice do not have to be sex matched. It is desirable that they are 8-10 weeks of age and weigh approximately 20 g.

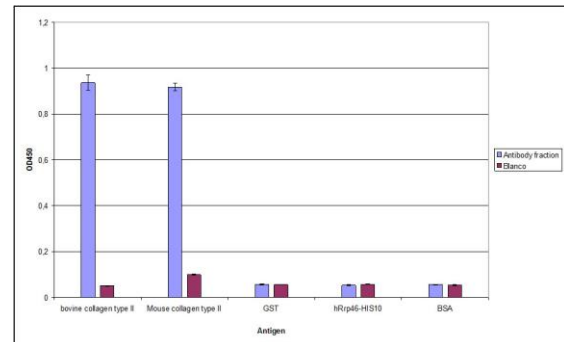


Figure 1: Specificity to mouse and bovine type II collagen have been determined in ELISA using anti-collagen-II antibody clone RC7. The Antibody has been diluted 12.000X from a stock of 10 mg/ml in MPBST. The antibody was tested on various recombinant protein substrates i.e. control protein, GST and BSA, 98% (Sigma).

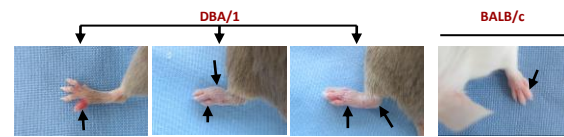


Figure 2: Mice where injected i.p. with 2.6 mg CAIA antibody mixture and followed during 7 days. Pictures were taken 3 days after injection.

Table1: Arthritis scoring method

| | |
|--|-------------|
| 1 - 2 Swollen Toes | 0.25 |
| 3 - 4 Swollen toes | 0.50 |
| Slightly Swollen footpad or ankle | 0.50 - 0.75 |
| Swollen Footpad or Ankle with | 1.00 |
| 1 - 2 Swollen Toes + Swollen Footpad or Ankle | 1.25 - 1.50 |
| Swollen Footpad + Swollen Ankle + Swollen Toes | 2.00 |

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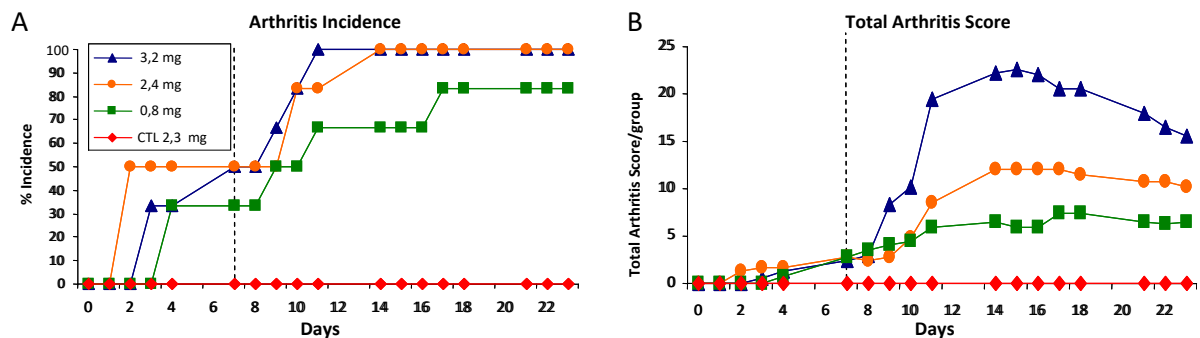


Figure 3: CAIA using 8 monoclonal antibodies. Arthritis incidence (A) and total arthritis score (B) are indicated. Groups of 6 DBA/1 mice were treated i.p. with 8 collagen specific monoclonal antibodies. Different concentrations of the antibody cocktail (0.8 mg, 2.4 mg and 3.2 mg) were used. An irrelevant control antibody (CTL) has been used at a 2.3 mg concentration. LPS (25 ug/mouse) was injected i.p. on day 7.

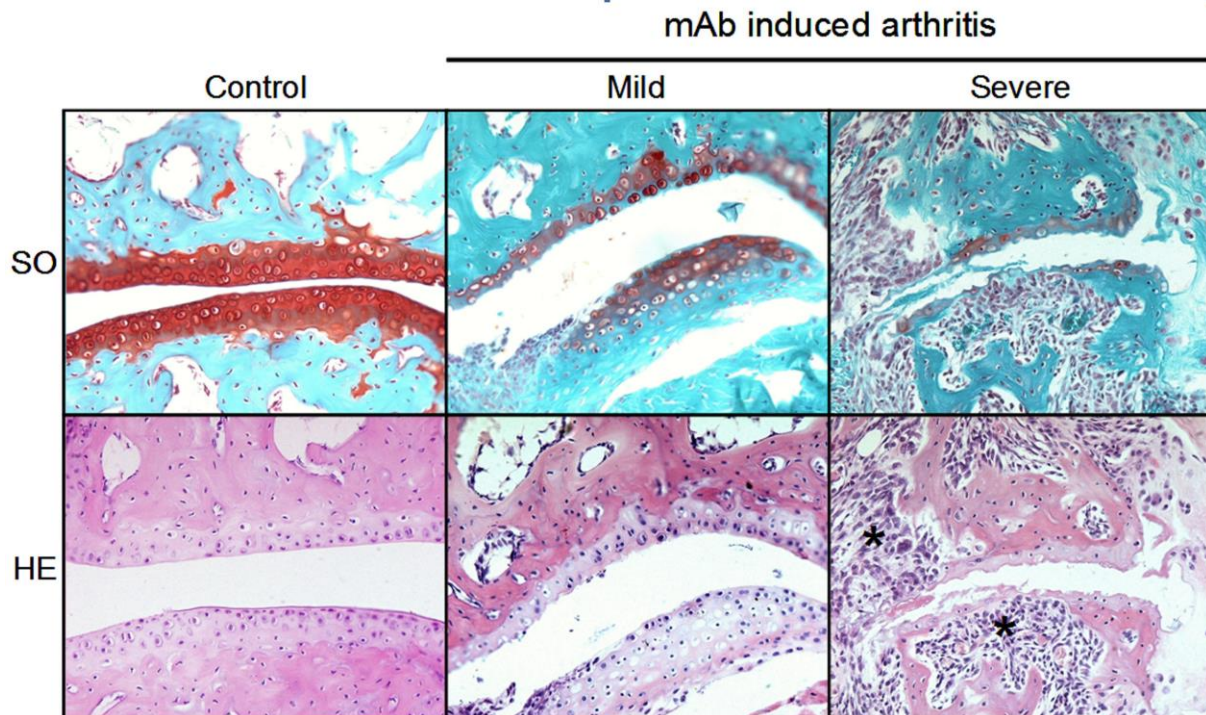


Figure 4: DBA/1 mice were treated i.p. with MQR-CAIA mix (2.3 mg total). An irrelevant control antibody (CTL) was used at the same concentration. LPS (25 ug/mouse) was injected i.p. on day 7. Pictures are histological preparations of hind paws 25 days after anti-collagen antibody injection. SO = Safranin O staining; Blue indicates bone, red indicates cartilage. HE = Haematoxylin-Eosin staining; * = inflammatory infiltrate.