Version 27-02/25

User's Manual

B-Crystallin ELISA Kit

REF DEIA-H032

5 x 96T

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

General Description

α-crystallins composed of ~20 kDa αA and αB subunits function asmajor water-soluble proteins accounting for almost 50% of total protein in the mammalian transparent eye lens, also existing in a variety of other tissues. The α-crystallin proteins are members of the small heat shock protein (sHsp) family, as their expression can be induced by heat and other stress insults in a variety of organisms. The α-crystallins possess structural and functional similarities and share sequence homology with Hsp25/27. The conserved α-crystallin domain participates in oligomer assembly, which is critical to their function in the prevention of irreversible protein aggregation.

Reagents And Materials Provided

1. αB-Crystallin Capture Antibody

One vial containing 156.25 µg lyophilized αB-Crystallin monoclonal antibody

2. αB-Crystallin Standard

One vial containing 500 ng lyophilized natural αB-Crystallin protein

3. αB-Crystallin Detection Antibody

One vial containing 31.25 µg lyophilized αB-Crystallin antibody

4. SA-HRP

One vial containing 12.5 µg lyophilized streptavidin conjugated to horseradish peroxidase

Buffer Formulations

1. Coating Buffer

10 mM sodium phosphate, 15 mM NaCl, pH 7.4

2. Blocking Buffer

10 mM sodium phosphate, 15 mM NaCl, 1.0% BSA, 1.0% sucrose, pH 7.4

3. Assay Buffer

100 mM sodium phosphate, 150 mM NaCl, 1.0% BSA, 0.1% Tween-20, pH 7.4

4. Wash Buffer

10 mM sodium phosphate, 15 mM NaCl, 0.1% Tween-20, pH 7.4

Materials Required But Not Supplied

1. RIPA Cell Lysis Buffer, or similar
2. 96-well high-binding polystyrene microtiter plates, or similar
3. Precision pipets
4. Microplate reader capable of reading at 450 nm
5. Microplate shaker

6. Phosphate buffered saline (PBS)
7. Tween-20*
8. Bovine Serum Albumin (BSA)
9. 3,3',5,5' tetramethylbenzidine (TMB) solution, or similar
10. 1N hydrochloric acid, suchas Stop Solution 2
11. Sucrose

*Tween is a registered trademark of ICL Americas

Storage

Store all components at 4°C. See page 3 for storage of reconstituted material.

Plate Preparation

1. Reconstitute αB-Crystallin Capture Antibody with 250 µL deionized water for a 250x stock. Use immediately, or make aliquots and freeze at -20°C for up to 3 months. For prolonged storage, aliquot and freeze at -80°C. Avoid repeated freeze/thaw cycles.
2. Dilute the stock 1:250 in Coating Buffer. Immediately dispense into 96-well microtiter plates using 100 µL of the diluted capture antibody per well. Seal the plate and incubate overnight at room temperature.
3. Aspirate each well to remove coating solution. Immediately add 200 µL Blocking Buffer per well. Seal the plate and incubate for at least 1 hour.
4. Aspirate each well to remove blocking solution. Plates may be used immediately or dried and store with desiccant at 41. Reconstitute αB-Crystallin Capture Antibody with 250 µL deionized water for a 250x stock. Use immediately, or make aliquots and freeze at -20°C for up to 3 months. For prolonged storage, aliquot and freeze at -80°C. Avoid repeated freeze/thaw cycles.
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4. Aspirate each well to remove blocking solution. Plates may be used immediately or dried and store with desiccant at 4°C.

Reagent Preparation

1. Recombinant αB-Crystallin Standard

Reconstitute αB-Crystallin Standard with 250 µL deionized water for a 50x stock. Aliquot and store at -20°C. For prolonged storage, aliquot and freeze at -80°C. Avoid repeated freeze/thaw cycles.

The recommended standard curve range is 40 ng/mL to 1.25 ng/mL, using 2-fold serial dilutions in Assay Buffer. Do not store diluted standard.

2. αB-Crystallin Detection Antibody

Reconstitute vial contents with 250 µL deionized water for a 250x stock. This solution may be stored at 4°C for up to 3 months. For prolonged storage, aliquot and freeze at -20°C. Avoid repeated freeze/thaw cycles.

For best results, reconstitute the Detection Antibody atthe time of plate coating and wait at least one day before freezing.

Dilute the stock 1:250 in Assay Buffer for a working solution. Do not store diluted antibody.

3. SA-HRP

Reconstitute vial contents with 250 µL deionized water for a 500x stock.

Store at 4°C for up to 3 months, or aliquot and freeze at -20°C for prolonged storage. Avoid repeated freeze/thaw cycles.

Dilute the stock 1:500 in Assay Buffer for a working solution. Do not store diluted conjugate.

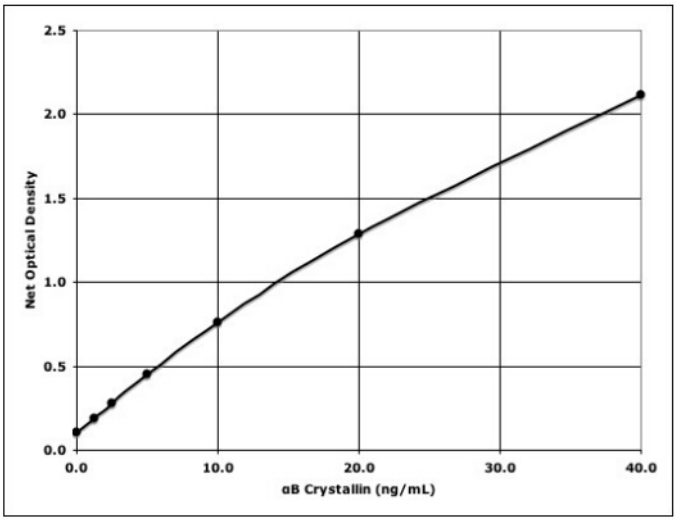
Assay Procedure

1. Pipet 100 µL of Assay Buffer into the control (0 ng/mL standard) wells.
2. Pipet 100 µL of standards and samples, prepared in Assay Buffer, to the bottom of the appropriate wells.
3. Seal the plate. Incubate for 1 hour on a plate shaker at room temperature.
4. Empty the contents of the wells and wash by adding 400 µL of Wash Buffer to every well. Repeat 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
5. Pipet 100 µL of the diluted detection antibody into each well, except the blank.
6. Seal the plate. Incubate for 1 hour on a plate shaker at room temperature.
7. Wash as above (Step 4).
8. Add 100 µL of the diluted conjugate to each well except the blank.
9. Seal the plate. Incubate for 30 minutes on a plate shaker at room temperature.
10. Wash as above (Step 4).
11. Pipet 100 µL of TMB solution into each well.
12. Seal the plate. Incubate for 30 minutes on a plate shaker at room temperature.
13. Pipet 100 µL 1N HCl into each well.
14. After blanking the plate reader against the substrate, read optical density at 450 nm. If the plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

Performance Characteristics

Typical Data

The results shown below are for illustration only and should not be used to interpret results from another assay.



Sensitivity

The sensitivity, or limit of detection, of this assay is 0.59 ng/mL. It was determined by interpolation at 2 standard deviations above the mean signal at background, using data from 5 standard curves.

Specificity

This assay detects human, mouse, rat, and bovine αB-Crystallin. Cross reactivity with γ-Crystallin and αA-Crystallin is 0.06% and 0.4%, respectively. There is no cross reactivity observed with ?βL-Crystallin, Hsp10, Hsp25 and Hsp27.

Dilutional Linearity

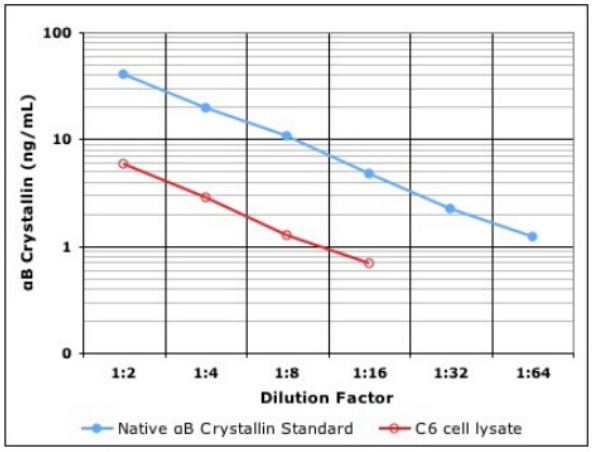
To determine possible interference from the sample matrix, the indicated sample types were serially diluted into assay buffer. The concentrations of αB-Crystallin were measured in the assay, and the results were analyzed to determine the range over which a linear response was obtained. These data may be used as a guideline to determine minimal recommended dilution (MRD) for similar samples.

Dilution Factor	HeLa CL	C6 CL	3T3 CL
1:2	32%	31%	40%
1:4	56%	67%	77%
1:8	92%	107%	101%
1:16	80%	103%	100%
1:32	100%	92%	---
1:64	---	100%	---

CL: Cell Lysate

Parallelism

Dose-response curves from cell lysates diluted into assay buffer (using the MRD) were compared to the recombinant αB-Crystallin standard curve. Parallelism indicates that the antibody-binding characteristics of the native and standard proteins are similar, allowing accurate determination of the analyte.



Precautions

1. If buffers other than those recommended are used in the assay, the end-user must determine the appropriate dilution and assay validation.
2. Pipet the reagents to the sides of the wells to avoid possible contamination.
3. Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.
4. Insufficient washing or residual wash buffer in the wells may cause variation in assay results.
5. Bring all reagents to room temperature for at least 30 minutes prior to opening.
6. All standards, controls, and samples should be assayed in duplicate.

Limitations

CD makes no warranty of any kind, expressed or implied, which extends beyond the description of the product in this brochure, except that the material will meet our specifications at the time of delivery. CD makes no guarantee of results and assumes no liability for injuries, damages or penalties resulting from product use, since the conditions of handling and use are beyond our control.