Product information

Background | Sandwich ELISA (Enzyme-linked Immunosorbent Assay) is used to quantitate cytoplasmic plant HSP70 concentration in plant extracts including *Medicago sativa, Linum usitatissimum, Zea mays*. The plate is coated with anti-plant HSP70 capture antibody.
In most eucaryotes hsp70 genes exist as part of a multigene family and are found in most cellular compartments including nuclei, mitochondria, chloroplasts, the endoplasmic reticulum and the cytosol. All HSP70 proteins, regardless of their location, bind particularly unfolded proteins. The universal ability of HSP70 proteins to undergo cycles of binding to and release from hydrophobic stretches of partially unfolded proteins determines their role in a great variety of vital intracellular functions such as protein synthesis, protein folding and oligomerization and protein transport.

Range: 1.563-100 ng/ml

Suggested standard curve points: 100 ng/ml, 50 ng/ml, 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, 3.125 ng/ml, 0 ng/ml

Sensitivity: 0.18 ng/ml

Application: this kit can be applied for quantitative detection of cytoplasmic plant HSP70 protein in up to 40 samples.

Incubation time: 30 minutes

Storage: 4°C for 6 months. Unused wells should be resealed with desiccant in the foil pouch provided and stored at 4°C until the kits expiry date.

NOTE: kit is for research purpose only. Not for human or animal disease diagnosis.

Kit components

Item	Specifications (96 wells)	Storage
Micro ELISA Plate	1 plate	4°C
Recombinant Hsp70 (Alfalfa) Standard	2 vials/lyophilized	4°C
Sample / Standard dilution buffer *	1 vial/50 ml	4°C
5X Hsp70 Extraction Reagent *	1 vial/10 ml	4°C
10X Wash Buffer Concentrate *	1 vial/100 ml	4°C
Anti-HSP70 Biotinylated Antibody *	1 vial/150 µl	4°C
Anti-HSP70 Biotinylated Antibody Diluent *	1 vial/13 ml	4°C
Streptavidin: HRP Concentrate	1 vial/ 50 µl	4°C
Streptavidin: HRP Diluent*	1 vial/13 ml	4°C
TMB Substrate	1 vial/13 ml	4°C
Stop Solution	1 vial/13 ml	4°C

*** This reagent contains preservative: ProClin 150, 0.06 %**

Important precautions before use

1. Please read provided instructions carefully before use.
2. The kit performs exclusively with provided reagents and may not performed as described if any reagent of procedure is replaced or modified.
3. Initial experiment using standards and a small number of samples is recommended.
4. All ELISA reagents must be at room temperature (20-25°C) before use.

5. TMB reagents should be stored in the dark, avoiding exposure to light. If TMB solution turns blue, it cannot be used.

6. Washing steps are of crucial importance to avoid false positive results.

7. We recommend duplicate well assay for standard and sample testing.

8. Use new disposable pipette tips for each transfer to avoid cross-contamination. Do not re-use tips and tubes to avoid cross contamination.

9. Use a new adhesive plate cover for each incubation step.

10. Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.

11. Individual components listed in the table on page 1 contain preservatives. Wear gloves while performing the assay. Please follow proper disposal procedures.

The following components are required but not supplied

1. Microplate reader (450 nm).
2. Single and multi-channel pipettes and tips.
3. Distilled or de-ionized water.
4. Graphing software for analyzing the data.
5. Automated plate washer or a wash bottle.
6. Polypropylene or polyethylene tubes to prepare samples – do not use polystyrene, polycarbonate or glass tubes for sample preparation.
7. Absorbing paper.
8. Additional reagents and materials for cell lysate and tissue extract preparation, including protease inhibitors.

Washing Method:
Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350 µl wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a total of three washes.

Automated Washing:
Aspirate all wells, then wash plate 3x with 350 µl wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute. Please note that automated plate washer can be easily contaminated thereby causing assay variability.

Sample Collection, Preparation and Storage:
Plant tissue: may be flash frozen, stored at -70°C. Please note that prolonged storage will influence quality of your sample.

Calculate the amount of 1x Extraction Reagent which is required. 4 ml of Extraction Reagent is to be used for every gram of tissue.

Prepare 1x Extraction Reagent by diluting 1 part of 5X Extraction Reagent with 4 parts of ice-cold ultra pure water. Example: If 5 ml of 1x Extraction Reagent are needed, dilute 1 ml of 5x Extraction Reagent with 4 ml of ultra pure water.

Use of other extraction reagents can lead to inaccurate results.

Add protease inhibitors like 0.1 mM PMSF or 1 µg/ml leupeptin, 1 µg/ml aprotinin, 1 µg/ml pepstatin or any other commercially available protease cocktail to the 1x Extraction Reagent.

Place the tissue in a mortar and add sufficient volume of liquid nitrogen to keep tissue covered.

Following liquid nitrogen evaporation the tissue will be thoroughly frozen.

Grind the frozen tissue to a powder with a pestle.

Add appropriate amount of ice-cold 1x Extraction Reagent including protease inhibitors.

Continue to homogenize the tissue with the pestle until its suspension becomes homogenous and transfer it to a fresh microcentrifuge tube and spin at 13 000 x g for 15 minutes at 4°C.

Transfer resulting supernatant to a fresh tube for analysis. Avoid disturbing the cell pellet at the bottom of each tube. Pellet is to be discarded. Harvested tissue supernatant is ready for analysis in the assay.

Tissue can be stored in single use aliquots at -70°C. Protein determination assay needs to be performed before the extracts are aliquoted and frozen.

Standard preparation (Alfaalfa *Medicago sativa* HSP70)
Add 0.5 ml of Standard and Sample Diluent to a vial with Standard for reconstitution and obtaining a final concentration of 100 ng/ml. Mix well.
Label seven (7) polypropylene or polyethylene tubes, one for each additional standard curve point: 50 ng/ml, 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, 3.125 ng/ml, 1.563 ng/ml and 0 ng/ml.

Pipet 250 µl of Standard and Sample Diluent into each tube.

Serial dilute the 100 ng/ml standard 1:1 with Standard and Sample Diluent by mixing 250 µl of the previous standard with 250 µl of Standard and Sample Diluent. Continue until standard value is 1.563 ng/ml.

Standard and Sample Diluent is used as the zero standard value.

1xWash Buffer Preparation
Prepare 1xWash Buffer by diluting 10xWash Buffer in ultra pure water. To prepare 1 liter of 1x Wash Buffer, dilute 100 ml of 10 x Wash Buffer into 900 ml of ultra pure water. Mix well. After reconstitution 1x Wash Buffer can be stored at 2-8°C for up to one month. 1x Wash Buffer cannot be used if it is visibly contaminated.

Biotinylated Antibody Working Solution Preparation
For every strip-well which is used (8 wells), prepare 1 ml of Biotinylated Antibody Working Solution by diluting Biotinylated Antibody Concentrate 1: 100 with Biotinylated Antibody Diluent.
If 12 ml of Biotinylated Antibody Working Solution is required (one whole plate), dilute 120 µl of Biotinylated Antibody Concentrated into 12 ml of Biotinylated Antibody Diluent. Mix well.

Performing the Assay

Determine the number of strips required. Leave these strips in the plate frame. Place unused strips in the foil pouch with desiccant and seal tightly. Unused strips should be stored at 2-8°C. Once the assay is completed, the plate frame should be kept for additional assays.

Use a Plate template to record the locations of the standards and unknown samples within the wells.

Add 100 µl of appropriately diluted standards or samples to each appropriate well. Run each standard, sample or blank in duplicate. Recommended sample dilution to start with

is 1: 8. This means to dilute 32 µl of sample in 224 µl of Standard and Sample Diluent. Mix well. If values for samples are not within the range of the standard curve, optimal sample dilutions need to be determined.

Carefully cover wells with a new adhesive plate cover. Incubate at 37°C for 2 hours.

Carefully remove adhesive plate cover, discard plate content and wash four (4) times with 1x Wash Buffer as described in the Plate Washing section below.

Washing Method
Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350 µl wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap
the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a total of three washes.

Automated Washing
Aspirate all wells, then wash plate 4x with 350 µl 1xWash Buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute. Avoid microbial contamination of equipment. Automated plate washers can easily become contaminated thereby causing assay variability.

Biotinylated Antibody Incubation
Calculate how much of Biotinylated Antibody Working Solution is required for strips being used. 100 µl of this solution is used/well.

Add 100 µl of Biotinylated Antibody Working Solution to each well containing standard, sample or blank. Mix well by gently tapping the plate several times.

Carefully attach a new adhesive plate cover. Incubate the plate for two (2) hours at 37°C.

Carefully remove the adhesive plate cover, discard content and wash four (4) times with 1x Wash Buffer as described in Plate Washing section above.

Streptavidin-HRP Incubation
Calculate how much of Streptavidin-HRP Working Solution is required for the number of strips being used. 100 µl of this solution is used/well.

Add 100 µl of Streptavidin-HRP Working Solution to each well containing standard, sample or blank.

Carefully attach a new adhesive plate cover. Incubate plate for 30 minutes at room temperature, 20-25°C.

Carefully remove the adhesive plate cover, discard plate content and wash four (4) times with 1x Wash Buffer as described in the Plate Washing section above.

TMB Substrate Incubation and reaction Stop
Prepare required amount of TMB Substrate and Stop Solution for the number of strips being used.

Caution when using TMB Substrate Solution:

1. Do not use a glass pipette to measure the TMB Substrate solution.
2. Do not cover the plate with aluminium foil or metalized mylar.
3. Do not return leftover of TMB Substrate back into the bottle.
4. Do not contaminate the unused TMB Substrate.
5. In case TMB Substrate Solution is blue, it cannot be used again.

Add 100 µl of TMB Substrate into each well.

Allow the enzymatic color reaction to develop at room temperature (20-25°C) in the dark for 30 minutes. Do not cover the plate with a plate sealer. The substrate reaction yields a blue solution.

After 30 minutes, reaction can be stopped by addition of 100 µl of Stop Solution to each well. Tap the plate gently to mix. The solution in the wells should change from blue to yellow.

Absorbance Measurement
Evaluate the plate within 30 minutes of reaction stop.

Wipe underside of wells with a lint-free tissue.
Measure the absorbance on an ELISA plate reader set at 450 nm.
Many plate readers come with data reduction software that plot data automatically. Alternatively a spreadsheet program can be used.

Calculations

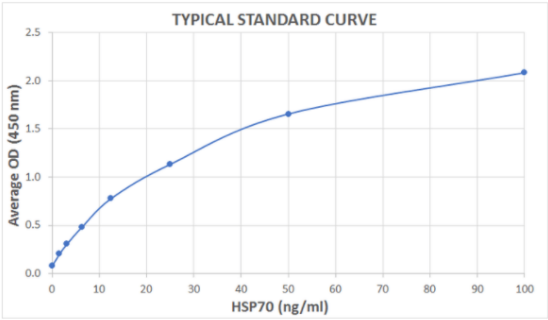
Duplicate absorbance values should be within 10 % of each other. Care should be taken when interpreting data with differences in absorbance values greater than 10 %.

Prepare a standard curve to determine the amount of HSP70 in an unknown sample. Plot the average absorbance obtained for each standard concentration on the vertical (Y) axis versus the corresponding HSP70 concentration on the horizontal (X) axis using graph paper or curve-fitting software.
Calculate the HSP70 concentration in unknown sample using the prepared standard curve. Determine the amount of HSP70 in each unknown sample by noting the HSP70

concentration (X axis) that correlates with the absorbance value (Y axis) obtained for the unknown sample.

Multiply the HSP70 concentration obtained by the dilution factor to determine the amount of HSP70 in the undiluted sample.

Example of a typical standard curve



This typical standard curve was generated using the plant HSP70 ELISA kit for demonstration purpose only. New standard curve has to be generated every time.

Limitations

1. Some samples may contain higher levels of interfering factors that can produce abnormal results.
2. If values for samples are not within the range of the standard curve, optimal sample dilutions need to be determined.
3. The use of assay reagents not provided in this kit can compromise the performance of this assay.
4. Do not mix components with reagents from other kits with different lot numbers.