

Bovine heat shock protein 70 (Hsp-70) ELISA Kit

Catalog # **BHS01-875**

Lot # 1Y2539-3

Product Description

Bovine heat shock protein 70 (Hsp-70) ELISA Kit is for the quantitative determination of Hsp-70 levels in bovine serum, plasma, culture media, or any biological fluid.

Assay Range and Sensitivity

Range: 0.312 ng/ml – 20 ng/ml

Recovery: 70 – 110 %

Intra-Assay CV: <8%

Inter-Assay CV: <12%

Sensitivity: 0.06 ng/ml

Components

Component Name	Storage Conditions	96 reactions
Plate Cover	R.T.	3
ELISA strip Microplate	2-8°C	8 x12
Standard (lyophilized)	2-8°C	2 vials
Biotinylated antibody (1:100)	2-8°C	120µl×1 bottle
HRP-Avidin (1:100)	2-8°C	130µl×1 bottle
HRP-Avidin diluent	2-8°C	12 ml×1 bottle
Antibody diluent	2-8°C	12 ml×1 bottle
Standard diluent	2-8°C	15 ml×1 bottle
Sample Diluent	2-8°C	15 ml×1 bottle
Washing buffer (1:25)	2-8°C	20ml×1 bottle
Color Reagent A (Avoid light)	2-8°C	10 ml×1 bottle
Color Reagent B (Avoid light)	2-8°C	1.5 ml×1 bottle
Stop Solution	2-8°C	10 ml×1 bottle

Storage and Stability

Store all reagents at 2-8°C. Product stored under these conditions should be stable for 2 weeks. For long term storage, store all reagents at -20°C for up to 12 months from production date.

Scientific Background

The microplate provided in this kit has been pre-coated with a monoclonal antibody specific to Hsp-70. Samples are added to the microplate wells and bind the specific antibody. Then a biotinylated polyclonal antibody specific to TGF-β2 is added to each well and incubated forming an antibody-antigen complex. Following a wash to remove any unbound reagent, HRP-avidin is added to each well

to bind Biotin-labeled antibody. After the unbound HRP-avidin has been washed away a color reagent is added to each well for coloration. Wells that contain Hsp-70 will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm.

Sample Preparation

Notes:

- Sample extraction and ELISA assay should be performed as soon as possible after sample collection. Samples should be extracted according to the relevant literature. If ELISA assay cannot be performed immediately, samples can be stored at -20°C. Repeated freeze-thaw cycles should be avoided.
- These kits cannot be used for samples with NaN₃ which can inhibit the activity of HRP.

Serum Samples

Collect whole blood. Allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during storage, the sample should be centrifuged again.

Plasma Samples

Collect whole blood into tubes with anticoagulant (EDTA or citrate). After incubation at room temperature for 10-20 minutes, centrifuge tubes for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during storage, the sample should be centrifuged again.

Urine, Cerebrospinal fluid, and Pleuroperitoneal Samples

Collect urine in aseptic tubes. Centrifuge for 20 min at 2,000-3,000 rpm and collect the supernatant carefully. If precipitates appear during storage, the sample should be centrifuged again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine samples.

Cell Samples

To detect cell secretions, collect culture supernatant into aseptic tubes. Centrifuge for 20 min at 2,000-3,000 rpm and collect the supernatant carefully. To detect intracellular components, dilute the cells to 1x10⁶ cells/ml with PBS (pH

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7.2-7.4). Destroy the cells by repeated freezing and thawing to release intracellular components. Centrifuge for 20 min at 2,000-3,000 rpm and collect the supernatant carefully. If precipitates appear during storage, the sample should be centrifuged again.

Tissue samples

Cut, weigh, and freeze tissue samples in liquid nitrogen. Store at -80°C for future use. Thaw at 4°C prior to use. Homogenize samples after adding PBS (pH 7.4). Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.

Procedure

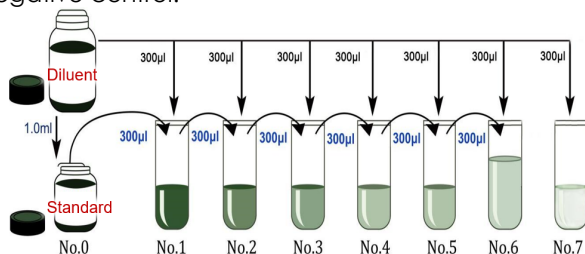
Notes:

- The kit should be equilibrated to room temperature before the assay. Remove any unneeded strips from the antibody-coated ELISA strip microplate, reseal them in self-sealing bag and keep at 4°C.
- Precipitates may appear in the concentrated wash solution. Please heat the solution to dissolve all the precipitates, this will not affect the results.
- To avoid cross-contamination, plate covers are for one-time use only.
- Keep Color Reagents A and B away from light.
- Stop solution is 1M sulfuric acid; pay close attention to safety when in use.
- All absorbance reading operations should be conducted strictly in accordance with the Microplate Reader manufacturer's instructions.
- All samples and waste products should be treated as infectious agents.
- Reagents from different lots should not be mixed.

30 min prior to start: Prepare the standard, biotinylated antibody, HRP-Avidin, and color reagent as described below. The standard dilutions, biotinylated antibody and HRP-Avidin cannot be reused for additional assaying.

Standard: Add 1.0ml Standard Diluent to the vial of lyophilized standard and wait for 30 min. After the standard is dissolved completely, mix it well and label No. 0 on the tube.

Standard Dilutions: Prepare 7 clean tubes and label No. 1, 2, 3, 4, 5, 6, 7. Add 300µL Standard Diluent into each tube. Pipette 300µl of diluent from No. 0 into No. 1 and mix well. Then Pipette 300µl of diluent from No. 1 into No. 2 and mix well. Repeat the serial dilution as shown in the figure. Do not pipette anything into tube No. 7 as it is the negative control.



Tube Number	Concentration of Standard Diluent
No.1	10ng/ml
No.2	5 ng/ml
No.3	2.5 ng/ml
No.4	1.25 ng/ml
No.5	0.625 ng/ml
No.6	0.312 ng/ml
No.7	Blank – 0 ng/ml

Biotinylated Antibody: Take the amount needed for the experiment, dilute with Antibody Diluent in a proportion of 1:100.

HRP-Avidin: Take the amount needed for the experiment, dilute with the HRP-Avidin Diluent in a proportion of 1:100.

Color Reagent: Mix 9 Color Reagent A and 1 Color Reagent B in a proportion of 9:1.

Step 1: Take out the required number of strips and allow them to reach room temperature. Reserve an empty well as a blank control.

Step 2: Add standards or samples to the corresponding wells (100µL for each well). Samples should be loaded onto the bottom without touching the well wall. Mix well by gently shaking. Seal with plate cover and incubate for 90 min at 37°C.

Step 3: Dilute the (1:25) wash solution with distilled water to a final concentration of 1X. The entire bottle may be diluted and stored at 2-8°C. Otherwise, note that approximately 5ml of 1X wash solution will be needed for each well.

Step 4: Carefully remove plate cover, aspirate the well contents, and refill with 450 – 500µl of the wash solution. Rest for 30 seconds then discard the wash solution. Repeat this washing procedure a total of 2 times.

Step 5: Add prepared Biotinylated Antibody to each well (100µL per well). Seal with plate cover and incubate for 60 min at 37°C. Wash the plate 3 times as described in Step 4.

Step 6: Add 100µl of prepared HRP-Avidin to each well except for the blank wells. Seal with plate cover and incubate for 30 min at 37°C. Wash the plate 5 times as in Step 4.

Step 7: Add 100µL of the prepared Color Reagent to each well (including the blank well), avoid light and incubate at 37°C. When the standard curve wells develop a dark color and there is a clear color gradient, the reaction can be terminated by using the Stop Solution. The time of the chromogenic reaction should take ~25min, do not go over 30 min. The color in the well

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should change from blue to yellow after adding the stop solution.

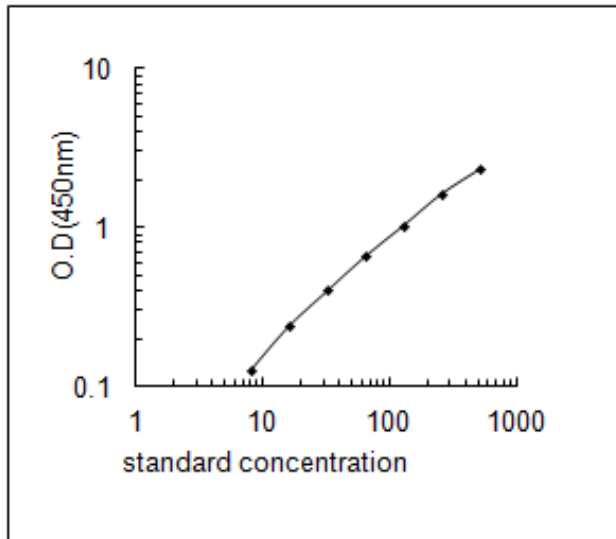
Step 8: Add 100 μ L Stop Solution to each well including the blank well. Mix well then Read OD (450nm). The OD value of the blank control well should be set to zero. The absorbance should be read within 10 minutes of adding the stop solution.

Calculation of Results:

1. Subtract the OD value of the blank from the OD of each sample well and standard. Draw the standard curve using this new value

2. Draw the standard curve. Set the concentration of standards as X- and OD value as Y-coordinates. Use a smooth line to connect each coordinate point of the standard values. The concentration of samples can be found by inputting the sample OD values into the line equation for the standard curve.

3. If the sample OD is higher than the highest standard in the standard curve, the sample should be diluted appropriately and then retested. Multiply the calculated concentration by the dilution factor to obtain the original concentration.



This diagram is for reference only

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SAFETY DATA SHEET

Article 1 - Product Identification

Product Name: Bovine heat shock protein 70(Hsp-70) ELISA Kit

Catalog # BHS01-875

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Supplier of Datasheet: SignalChem Diagnostics Inc.
 Street Address: 190-13160 Vanier Place
 City, Prov. Postal Code: Richmond, BC, V6V 2J2
 Country: Canada
 Emergency Phone: 1-888-606-3424 (Toll free)
 1-778-326-0223 (local)

Article 2 - Hazard Identification

- **WHMIS Classification:** Not WHMIS controlled.
- **GHS classification:** Not GHS classified.
- **Hazard Pictograms:** No labeling applicable.
- **Signal words:** None.
- **Hazard statements:** None.
- **Precautionary statements:** Wear protective gloves/protective clothing/eye protection/ face protection. Avoid breathing dust. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- **Other hazards:** none known.

Article 3 – Composition/Information on Ingredients

Component: Standard (lyophilized)

Chemical Characterization: Mixtures.

Description: No hazardous substances in concentrations to be declared.

Component: Biotinylated antibody (1:100)

Chemical Characterization: Mixtures.

Description: This product contains the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Glycerol	1,2,3-Propanetriol	56-81-5	50%

Component: HRP-Avidin (1:100)

Chemical Characterization: Mixtures.

Description: This product contains the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Glycerol	1,2,3-Propanetriol	56-81-5	50%

Component: HRP-Avidin diluent

Chemical Characterization: Mixtures.

Description: This product contains the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Sodium Azide	N3Na	26628-22-8	0.01%

Component: Antibody diluent

Chemical Characterization: Mixtures.

Description: This product contains the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Sodium Azide	N3Na	26628-22-8	0.01%

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Component: Standard diluent

Chemical Characterization: Mixtures.

Description: This product contains the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Sodium Azide	N ₃ Na	26628-22-8	0.015%

Component: Sample Diluent

Chemical Characterization: Mixtures.

Description: This product contains the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Sodium Azide	N ₃ Na	26628-22-8	0.015%

Component: Washing buffer (1:25)

Chemical Characterization: Mixture

Description: No hazardous substances in concentrations to be declared

Component: Color Reagent A

Chemical Characterization: Mixtures.

Description: This product contains the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Hydrogen peroxide	H ₂ O ₂	7722-84-1	0.16%

Component: Color Reagent B

Chemical Characterization:

Description: This product contains the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
TMB	3,3',5,5'-Tetramethylbenzidine	54827-17-7	0.16%

Component: Stop solution

Chemical Characterization:

Description: This product contains the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Sulfuric Acid	H ₂ SO ₄	7664-93-9	<1%

Article 4 – First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** In case of irritation by inhaling this product, move affected person to fresh air and await recovery. If irritation persists, seek immediate medical attention. If casualty cannot breathe, give artificial respiration, and seek immediate medical attention.
- **After skin contact:** Immediately wash with soap and plenty of water and rinse thoroughly. Consult a physician.
- **After eye contact:** Rinse opened eyes with plenty of water for at least 15 minutes. Remove contact lenses, if present and easy to do so. Consult a physician.
- **After swallowing:** Not expected to present a significant ingestion hazard under anticipated conditions of normal use. If you feel unwell, seek medical advice.

Article 5 - Fire-fighting Measures

- **Suitable extinguishing media:** Use water spray, extinguishing powder, carbon dioxide, or other appropriate measure that is suitable to the environment.
- **Specific hazards arising from the substance or mixture:** None known.
- **Special protective equipment and precautions for fire-fighters:** Self-contained breathing apparatus if necessary.

Article 6 – Accidental Release Measures

- **Personal precautions, protective equipment, and emergency procedures:** Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- **Environmental precautions:** Do not allow to enter drains.
- **Methods and materials for containment and cleaning up:** Absorb on sand or vermiculite and place in closed containers for disposal.

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Article 7 - Handling and Storage

- **Precautions for safe handling:** Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- **Conditions for safe storage:** Store in a dry and well-ventilated place. Keep container upright and tightly closed.

Article 8 - Exposure Controls/Personal Protection

- **Components with limit monitoring values at workplace:** N/A
- **Appropriate engineering controls:**
Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.
- **Individual protection measures:**
Respiratory protection:
Use appropriate respirator if there is inadequate ventilation by following the government standards.
Hand protection:
Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.
Eye/face protection:
Safety goggles with side-shields approved under appropriate government standards.
Skin/body protection:
Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

Article 9 – Physical and Chemical Properties

Color Reagent A, Color Reagent B, Sample Diluent:

Appearance: Liquid.	Danger of explosion: Product does not present an explosion hazard.
Odour/Odour Threshold: Not determined.	Explosion limits: Not determined.
pH: Not determined	Decomposition temperature: Not available.
Melting point/freezing point: Not determined.	Vapor pressure at 20 °C: Not determined.
Boiling point/Boiling range: Not determined	Density: Not determined.
Flash point: Not determined.	Relative density: Not determined.
Flammability (solid, gaseous): Not determined.	Vapor density: Not determined.
Ignition temperature: Not determined.	Evaporation rate: Not determined.
Auto-igniting: Product is not self-igniting.	Solubility in / Miscibility with Water: Fully miscible.

30X Wash Solution, HRP-Conjugate Reagent, Stop Solution:

Appearance: Clear liquid.	Danger of explosion: Product does not present an explosion hazard.
Odour/Odour Threshold: Not determined.	Explosion limits: Not determined.
pH: Not determined	Decomposition temperature: Not available.
Melting point/freezing point: Not determined.	Vapor pressure at 20 °C: Not determined.
Boiling point/Boiling range: Not determined	Density: Not determined.
Flash point: Not determined.	Relative density: Not determined.
Flammability (solid, gaseous): Not determined.	Vapor density: Not determined.
Ignition temperature: Not determined.	Evaporation rate: Not determined.
Auto-igniting: Product is not self-igniting.	Solubility in / Miscibility with Water: Fully miscible.

Article 10 - Stability and Reactivity

- **Reactivity:** Stable under recommended transport and storage conditions.
- **Chemical stability:** Stable under recommended transport and storage conditions.
- **Possible hazardous reactions:** No dangerous reactions known.
- **Conditions to avoid:** Heat and moisture.
- **Incompatible materials:** Not determined.
- **Hazardous decomposition products:** Not determined.

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Article 11 - Toxicological Information

- **Acute toxicity:** Not available.
- **LD/LC50:** Not available.
- **Skin corrosion/irritation:** Not available.
- **Serious eye damage/eye irritation:** Not available.
- **Respiratory or skin sensitization:** Not available.
- **Germ cell mutagenicity:** Not available.
- **Carcinogenicity:** No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- **Reproductive toxicity:** Not available.
- **Teratogenicity:** Not available.
- **Specific target organ toxicity - single exposure/ - repeated exposure (GHS):** Not available.
- **Aspiration hazard:** Not available.
- **Potential health effects:**
Inhalation: May be harmful if inhaled. May cause respiratory tract irritation.
Ingestion: May be harmful if swallowed.
Skin: May be harmful if absorbed through skin. May cause skin irritation.
Eyes: May cause eye irritation.
- **Signs and Symptoms of Exposure:**
Prolonged or repeated exposure can cause: Nausea, Dizziness.
- **Synergistic effects:** Not available.

Article 12 - Ecological Information

- **Eco-toxicity:** No data available.
- **Biodegradability:** Not applicable.
- **Bio-accumulative potential:** Not applicable.
- **Mobility in soil:** Not applicable.
- **PBT and vPvB assessment:** Not applicable.
- **Other adverse effects:** Not applicable.

Article 13 - Disposal Considerations

- **Disposal methods:** In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- **Contaminated packaging:** Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

Article 14 - Transport Information

- **DOT:** Not dangerous goods.
- **IMDG:** Not dangerous goods.
- **IATA:** Not dangerous goods.

Article 15 - Regulatory Information

- **WHMIS Classification:** Non-hazardous.
- **GHS label elements:** Not applicable.
- **Signal word:** Not applicable.
- **Hazard statements:** Not applicable.

Article 16 - Other Information

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.

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