Datasheet for ABIN625462

# HSP70 ELISA Kit

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## Overview

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| Quantity: | 96 tests |
| Target: | HSP70 |
| Reactivity: | Mouse, Rat, Human |
| Method Type: | Sandwich ELISA |
| Detection Range: | 2-600 ng/mL |
| Minimum Detection Limit: | 2 ng/mL |
| Application: | ELISA |

## Product Details

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| Purpose: | Human HSP70 ELISA Kit for cell culture supernatants, Heparin and/or EDTA treated plasma, and serum samples. Citrate is not recommended. |
| Sample Type: | Plasma, Cell Culture Supernatant, Serum |
| Analytical Method: | Quantitative |
| Detection Method: | Colorimetric |
| Specificity: | This ELISA antibody pair also can recognize mouse and rat HSP70 |
| Sensitivity: | 2 ng/mL |
| Characteristics: | • Strip plates and additional reagents allow for use in multiple experiments<br>• Quantitative protein detection<br>• Establishes normal range<br>• The best products for confirmation of antibody array data |

## Product Details

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| Components: | • Pre-Coated 96-well Strip Microplate<br>• Wash Buffer<br>• Stop Solution<br>• Assay Diluent(s)<br>• Lyophilized Standard<br>• Biotinylated Detection Antibody<br>• Streptavidin-Conjugated HRP<br>• TMB One-Step Substrate |
| Material not included: | • Distilled or deionized water<br>• Precision pipettes to deliver 2 µL to 1 µL volumes<br>• Adjustable 1-25 µL pipettes for reagent preparation<br>• 100 µL and 1 liter graduated cylinders<br>• Tubes to prepare standard and sample dilutions<br>• Absorbent paper<br>• Microplate reader capable of measuring absorbance at 450nm<br>• Log-log graph paper or computer and software for ELISA data analysis |

## Target Details

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| Target: | HSP70 |
| Alternative Name: | HSP70 (HSP70 Products) |
| Background: | The Human HSP70 (Heat Shock Protein 70) ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human HSP70 in serum, plasma (Collect plasma using EDTA and heparin as an anticoagulant. Citrate are not recommended), cell culture supernatants and urine. This assay employs an antibody specific for human HSP70 coated on a 96-well plate. Standards and samples are pipetted into the wells and HSP70 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human HSP70 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of HSP70 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm. Reproducibility: Intra-Assay: CV<10% Inter-Assay: CV<12%. |
| Gene ID: | 3308 |
| UniProt: | P34932 |

## Application Details

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| Application Notes: | Recommended Dilution for serum and plasma samples2 fold |
| Sample Volume: | 100 µL |
| Plate: | Pre-coated |
| Protocol: | 1. Prepare all reagents, samples and standards as instructed in the manual.<br>2. Add 100 µL of standard or sample to each well.<br>3. Incubate 2.5 h at RT or O/N at 4 °C.<br>4. Add 100 µL of prepared biotin antibody to each well.<br>5. Incubate 1 h at RT.<br>6. Add 100 µL of prepared Streptavidin solution to each well.<br>7. Incubate 45 min at RT.<br>8. Add 100 µL of TMB One-Step Substrate Reagent to each well.<br>9. Incubate 30 min at RT.<br>10. Add 50 µL of Stop Solution to each well.<br>11. Read at 450 nm immediately. |
| Reagent Preparation: | 1. Bring all reagents and samples to room temperature (18 - 25°C) before use. 2. Sample dilution: If your samples need to be diluted, Assay Diluent C (Item L) is used for dilution of serum/plasma/culture supernatants/urine. 3. Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water before use. 4. Preparation of standard: Briefly spin the vial of Item C. Add 400 µl Assay Diluent C (Item L) into Item C vial to prepare a 600 ng/ml standard solution. Dissolve the powder thoroughly by a gentle mix. Pipette 400 µl Assay Diluent C into each tube. Use the 600 ng/ml standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Gently vortex to mix. Assay Diluent C serves as the zero standard (0 ng/ml). 5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer. 6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Assay Diluent B (Item E) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in step 4 of Part VI Assay Procedure. 7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 25,000-fold with 1x Assay Diluent B (Item E). For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 2 µl of HRP-Streptavidin concentrate into a tube with 198.0 µl 1x Assay Diluent B to prepare a 100-fold diluted HRP-Streptavidin solution (do not store the diluted solution for next day use). Mix through and then pipette 40 µl of prepared 100-fold diluted solution into a tube with 10 ml 1x Assay Diluent B to prepare a final 25,000 fold diluted HRP-Streptavidin solution. |

## Application Details

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| Assay Procedure: | 1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate. 2. Add 100 µl of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking. 3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels. 4. Add 100 µl of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking. 5. Discard the solution. Repeat the wash as in step 3. 6. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking. 7. Discard the solution. Repeat the wash as in step 3. 8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking. 9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately. |
| Calculation of Results: | Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points. |
| Restrictions: | For Research Use only |

## Handling

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| Storage: | -20 °C |
| Storage Comment: | The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. |
| Expiry Date: | 6 months |

## Publications

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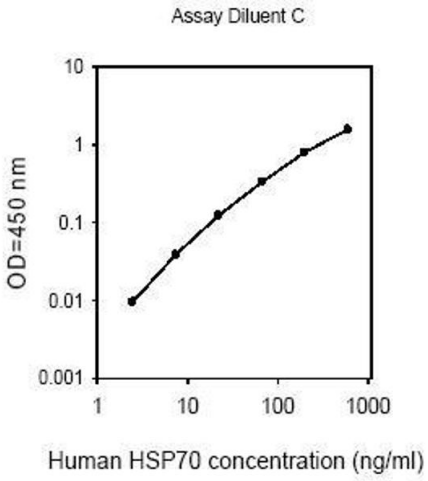
| Product cited in: | Pei, Pan, Zhu, Ding, Liu, Lv, Zou, Luo: "Gemcitabine-treated pancreatic cancer cell medium induces the specific CTL antitumor activity by stimulating the maturation of dendritic cells." in: **International immunopharmacology**, Vol. 19, Issue 1, pp. 10-6, (2014) (PubMed). |




## ELISA

**Image 1.**