HIS TAG ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HIS TAG CONCENTRATIONS IN RECOMBINANT HIS TAGGED PROTEIN SOLUTION AND THE SUPERNATANT OF ANIMAL FREE CELL CULTURES.

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ AND CHECK ALL ITEMS OF EACH KIT BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PURCHASE INFORMATION:

ELISA NAME	**HIS TAG ELISA KIT**
Catalog No.	**SK00779-06**
Lot No.	
Formulation	**96 T (8 wells x 12 strips)**
Standard range	**1.6 – 200 ng/mL**
Dynamic range	**1.6 – 200 ng/mL**
Sensitivity	**~0.3 ng/mL**
Sample Volume	**50 µL**
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	**Recombinant His Tagged Protein Solution, the supernatant of animal free cell culture**
Specificity	**6 x or 5 x His Tag on C-terminal or N-Terminal**
Intra-assay Precision	**4-6%**
Inter-assay Precision	**8-10%**
Storage	**2-8°C for 1 month. See page 2-3 for more information**

Order Contact:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Email: Info@AvisceraBioscience.com
www.AvisceraBioscience.net

INTRODUCTION

The His Tag ELISA Kit employs the quantitatively competitive enzyme immunoassay technique in which poly His Tag present in samples or His Tagged Protein Standards compete with a fixed amount of His Tagged proteins on the pre-coated microplate, for the sites on Anti His Tag monoclonal antibody horseradish-peroxidase (HRP) conjugate. During the incubation period, the Monoclonal Antibody HRP Conjugate specific for His Tag binds to the microplate. Following a wash to remove any unbound monoclonal antibody HRP conjugate, standard and samples, a TMB substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when stop solution is added. The intensity of the color measured is in inverse proportion to the amount of His Tag bound in the initial step. The higher the concentration of the His-tagged protein in solution is, the less the antibody bound to the plate will be.

This Aviscera Bioscience His Tag ELISA Kit, a 45 minutes competition ELISA, is beneficial for fast and high throughput detection of His-tagged proteins, quickly optimize protein expression by monitoring the His-tagged proteins level.

LIMITATIONS OF THE PROCEDURE

_ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_ The kit should not be used beyond the expiration date on the kit label.
_ Do not mix or substitute reagents with those from other lots or sources.
_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_ If samples generate values higher than the highest standard, dilute the samples with Dilution Buffer and repeat the assay.
_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
_Some vials contain small quantities of material, therefore centrifuge before use.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
His Tag-Microplate - 96 well microplate pre-coated with His Tagged Proteins.	**779-06-01**	**1 plate**
His Tag Standard – 1000 ng/vial of recombinant His Tagged Protein in a buffered protein base with preservative; lyophilized.	**779-06-02**	**1 vial**
Anti His Tag Monoclonal Antibody HRP Conjugate – 80 µL of 80-fold concentrated purified monoclonal antibody HRP conjugate against His Tag;.	**170-06-03**	**1 bottle**
Dilution Buffer – 45 mL of buffered surfactant protein based solution with preservative. Ready to use.	**DB11**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.25M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 – 8 °C for up to 1 month. For longer storage up to 10 months, unopened Standard and Dilution Buffer DB11 should be stored at -20 °C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard stock SHOULD BE STORED at -20 °C or -70 °C for up to one month.
Anti His Tag Monoclonal Antibody HRP Conjugate 80-fold concentrated solution **(protect from light)** and TMB Substrate Solution should be stored only at 2 – 8 °C for up to 10 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 10 months at 2 – 8 °C. Do not use kit past expiration date.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm and or 650nm.
- Microplate shaker (350-400rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE PREPARTION

Minimize concentration of certain reagents in the sample. For some reagents may interfere with test result. Filter the sample and wash by PBS (pH 7.4) or TBS (pH 7.4) on micro-filter centrifuge. Dialysis the sample in PBS or TBS. Samples should not contain any particles / precipitates. Filter the sample or centrifuge as necessary to remove insoluble materials. For best results, the sample should be adjusted to neutral pH (6.8-7.4). For cell cultures supernatant, must use ***animal serum free media***.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.
Optimal dilutions should be determined by each laboratory for each application with a pretest. Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

His Tag Standard - Refer to vial label for reconstitution volume. Reconstitute the **standard** with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 1000 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 200 µL of Dilution Buffer into tubes #1 to #4. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **200 ng/mL** standard serves as the high standard.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0mL	1000 ng/ml
# 1	50µl of stock	200µl	200 ng/ml
# 2	50µl of 1	200µl	40 ng/ml
# 3	50µl of 2	200µl	8 ng/ml
# 4	50µl of 3	200µl	1.6 ng/ml

Anti His Tag Antibody-HRP Conjugate - Transfer 80 µL of 80-fold concentrated **Anti His Tag Antibody-HRP Conjugate** stock solution to 6.32 mL of **Dilution Buffer** to prepare working solution. **Note:** 1x working solution of His Tag Antibody-HRP Conjugate should be freshly prepared and used within a few hours **(protect from light)**.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicate.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Leave wells H2, H3 as Blank. Add 100 ul of Dilution Buffer on Blank well. **DO NOT ADD ANY ANTIBODY OR STANDARD SOLUTION INTO BLANK WELLS.**
4. Set A2, A3 as total binding (TB). Add 50 µL per well of Dilution Buffer.
5. Add 50 µL per well of **standard solutions** from #4 to #1 (reverse order of serial dilution) to the

appropriate wells (B2, B3 to F2, F3). Add 50 µL per well of **samples** into appropriate wells.

6. Add 50 µL per well of 1x Working Solution of His Tag Antibody-HRP Conjugate into total binding, standard and sample wells. Cover with plate sealer and incubate on microplate shaker (350-400rpm) at room temperature for 30 min
7. Aspirate wells and wash 4 times with 300 µL of **1x Wash Buffer**. Blot plate on absorbent paper to remove any residual buffer.
8. Add 100 µL of **Substrate Solution** to each well. Incubate on microplate shaker (350-400rpm) for 12-15 minutes at room temperature. **Protect from light**.
9. Add 100 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. It is recommended to add the stop solution when the Total Binding or the lowest standard has developed a dark blue color.
12. Determine the optical density of each well within 3 minutes using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average Blank optical density. It is recommended to use software capable of generating a four parameter logistic (4-PL) curve-fit. The standard curve shows relationship between standard concentrations and corresponding O.D. absorbances. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION

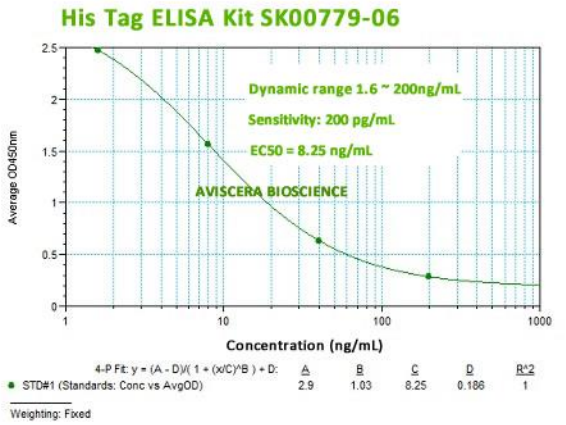
This immunoassay is calibrated against a highly purified recombinant His Tagged Protein MW at 11.7 KD.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

LAYOUT	STANDARD (NG/ML)	AVERAGE OD450NM (CORRECTED)
BLANK		0 (0.045)
STD1	200	0.275
STD2	40	0.625
STD3	8	1.502
STD4	1.6	2.519
TOTAL BINDING	0	2.709

IC50= 8.25ng/mL



SPECIFICITY

This assay recognizes 6 x or 5x His Tag on C-terminal or N-Terminal recombinant Proteins.

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 50 µL of standard, samples, to the well. Add 50 µL of Dilution Buffer to Total Binding well. Add 50 µL of 1x Antibody HRP Solution to each well used, except blanks. Incubate 30 min on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL Substrate Solution to each well. Incubate 12 -15 min on plate shaker at RT. **Protect from light.**
↓
Add 100 µL Stop Solution to each well. Read 450nm within 3 min.

Reagent compatibility

Reagent	Recommended Use
PBS, pH 6.8-7.4	Fully compatible
PBST, pH 6.8-7.4	Fully compatible
TBS, pH 7.4 -8.0	Fully compatible
TBST, pH 7.4- 8.0	Fully compatible
Tween-20	< 1%
SDS	<0.1%
Triton X-100	<0.1%

Sample solution should not contain TCEP, DTT, Urea, Guanidine HCl, Guanidine HCl, SDS (>1%) and Triton X-100 (> 1%) that may reduce activity of Anti His Tag Antibody HRP conjugate. Dialysis samples against PBS or microfilter samples by micro-centrifuge and wash by PBS.