KAMIYA BIOMEDICAL COMPANY

Human Short Talin Chemiluminescent Immunoassay (CLIA)

For the quantitative determination of free short talin in human serum

Cat. No. KT-1883

For Research Use Only. Not for use in diagnostic procedures.

PRODUCT INFORMATION

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INTENDED USE

The Human Short Talin (S-Talin) Chemiluminescent Immunoassay (CLIA) is for the quantitative determination of free S-Talin fragment present in human serum in the range from 31 ng/mL to 2,000 ng/mL.
This kit is for research use only. Not for use in diagnostic procedures.

INTRODUCTION

S-Talin is a cytoskeletal protein which is involved in integrin mediated signal transduction (1). Recently it has been reported that in rheumatoid arthritis (RA), a short portion of the cytoplasmic domain of S-Talin is cleaved and released into the circulation (2). Furthermore, quantitation of the circulating levels of the S-Talin peptide has been demonstrated to be a better marker of RA than the more traditional markers such as anti-CCP (ACCP) antibody (3).

ASSAY PRINCIPLE

The S-Talin test is based on the principle of a solid phase enzyme-linked sandwich immunosorbent assay (4, 5). The assay system utilizes a specific monoclonal antibody directed against a distinct epitope on the S-Talin molecule, and is coated on the microtiter wells for the solid phase immobilization of the S-Talin. A second monoclonal antibody directed against a different epitope on the S-Talin and conjugated with horse radish peroxidase (HRP) is used for detecting the S-Talin. The test sample (serum) is allowed to react with the capture antibody which immobilizes the S-Talin present in the sample. Following washing, HRP conjugated detection antibody is added to wells and allowed for the bound S-Talin to form the sandwich with the two antibodies. Following another wash, a chemiluminescent substrate of HRP is added and incubated. The resulting luminescence is measured spectrophotometrically using a luminometer. The concentration of S-Talin is directly proportional to the relative luminescent units (RLU) of the test sample.

COMPONENTS

- Antibody-Coated Microtiter wells (1 break-apart plate, 96 wells) coated with monoclonal anti-S-Talin antibody.
- Calibrator Set containing lyophilized S-Talin calibrator (2,000 ng/vial, 3 vials/kit)
- Sample diluent (6 mL)
- Calibrator diluent (12 mL of human serum with preservatives)
- HRP Conjugated monoclonal antibody reagent (12 mL)
- Wash buffer concentrate (25 mL of 20x PBST)
- Chemiluminescent substrate A and B (6 mL each)

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes: 50 µL, 100 µL, and 1.0 mL
- Disposable pipette tips
- De-ionized water
- Vortex mixer or equivalent
- Plate shaker
- Absorbent paper or paper towels
- Microtiter luminometer capable of measuring luminescent signal

STORAGE

- Store the kit at 4℃ upon receipt. Refer to the package label for the expiration date.
- The opened and used reagents are stable until the expiration date if stored properly at 4℃.

• Keep antibody coated microtiter plate dry in a sealed bag with desiccant to minimize exposure to moisture.

REAGENT PREPARATION

Calibration curve:

• In a holder set up seven 1.5 mL tubes such as eppendorf tubes and label them 2 to 8.
• Add 200 µL of calibrator diluent into each of the 7 tubes.

• Add 1 mL of calibrator diluent in one of the vials containing S-Talin calibrator, mix by vortexing to get 2,000 ng/mL S-Talin solution. Label this vial as #1.

• Make a 2-fold serial dilution of the 2,000 ng/mL S-Talin solution by transferring 200 µL from tube #1 to #2, #2 to #3 and all the way to #7, to get 1,000, 500, 250, 125, 62.5, and 31 ng/mL S-Talin solutions. Note tube #8 has only the calibrator diluent and is the 0 ng/mL S-Talin control.

Wash buffer:

Dilute the entire 25 mL of the 20x wash buffer concentrate to 500 mL with distilled water in a bottle and store it capped. The 1x wash buffer is good for 6 months at room temperature.

SAMPLE COLLECTION AND PREPARATION

• The use of serum samples is required for this test.

• Serum should be collected using standard techniques.

• Samples which cannot be assayed within 6 hours after collection may be frozen at –20℃ or lower, and will be stable for up to six months.
• Samples should not be repeatedly frozen and thawed prior to testing. DO NOT store in “frost free” freezers, which may cause occasional thawing.

• Specimens which have been frozen, and those which are turbid and/or contain particulate matter, must be centrifuged prior to use.

ASSAY PROTOCOL

• Secure the desired number of coated wells in the holder.

• Dispense 50 µL of S-Talin calibrators, test samples and controls into the appropriate wells (An example of the layout is shown in Figure 1 below).

Well ID	Talin Standard (ng/ml)		Samples			
	1	2	3	4	5	6
A	0	0	Sample 1	Sample 1	Sample 9	Sample 9
B	31	31	Sample 2	Sample 2	and so on	
C	62.5	62.5	Sample 3	Sample 3		
D	125	125	Sample 4	Sample 4		
E	250	250	Sample 5	Sample 5		
F	500	500	Sample 6	Sample 6		
G	1000	1000	Sample 7	Sample 7		
H	2000	2000	Sample8	Sample8		

Figure 1: A typical plate layout of the S-Talin CLIA

• Dispense 50 µL of Sample Diluent into each well.

• Thoroughly mix for 20-30 seconds on a plate shaker. It is very important to mix completely.
Note: It is recommended that all samples including the calibrators are run in duplicate at the minimum.
• Incubate at 37℃ (or at room temperature if a 37℃ shaker is not available) for 60 minutes on a plate shaker.

• Remove the incubation mixture by flicking plate contents into a waste container. (Alternatively a plate/strip washer can be used).

• Wash the microwells 3-4 times with 300 µL of 1x wash buffer/well.

• Add 100 µL/well of the HRP-conjugated antibody reagent.

• Incubate at 37℃ (or at room temperature if a 37℃ shaker is not available) for 60 minutes, on a plate shaker.

• Remove the incubation mixture by flicking plate contents into a waste container. (Alternatively a plate/strip washer can be used).

• Wash the microwells 4-5 times with 300 µL of 1x wash buffer/well.

• Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash solution.

• While the samples are incubating prepare required volume of the chemiluminescent substrate by mixing equal volume of reagents A and B in a separate tube.

• Dispense 100 µL of the chemiluminescent substrate solution into each well. Gently mix for 5 seconds.

• Incubate at room temperature for 5 minutes.

• Read the luminescence in each well using a luminometer plate reader.

CALCULATIONS

• Calculate the mean luminescence (RLU) for each set of reference calibrators, controls and samples.

• Subtract RLU for 0 ng/mL S-Talin from all the values.

• Construct a calibration curve by plotting the mean RLU obtained for each reference calibrator against its concentration in ng/mL, with RLU values on the vertical or Y axis, and concentrations on the horizontal or X axis.

• Use the mean RLU values for each sample to determine the corresponding concentration of S-Talin in ng/mL from the calibration curve.

• Note: Many plate readers come with built-in software for data analysis, which can be used for processing and analyzing the data.

EXAMPLE CALIBRATION CURVE

A typical calibration curve is shown in Figure 2 below. This calibration curve is for illustrative purpose only, and should not be used to calculate unknowns. Each laboratory should obtain its own data and calibration curve.

Talin (ng/ml)	RLU			Avg RLU	Net RLU
0	581	591	557	576	0
62.5	1236	917	993	1049	472
125	2248	3155	1995	2466	1890
250	5631	5995	6348	5991	5415
500	13543	14066	16320	14643	14067
1000	30880	25080	25561	27174	26597
2000	49974	50942	56026	52314	51738

Table 1: Typical results showing (triplicate) RLU, average RLU and net RLU (after background subtraction) for each S-Talin concentration.

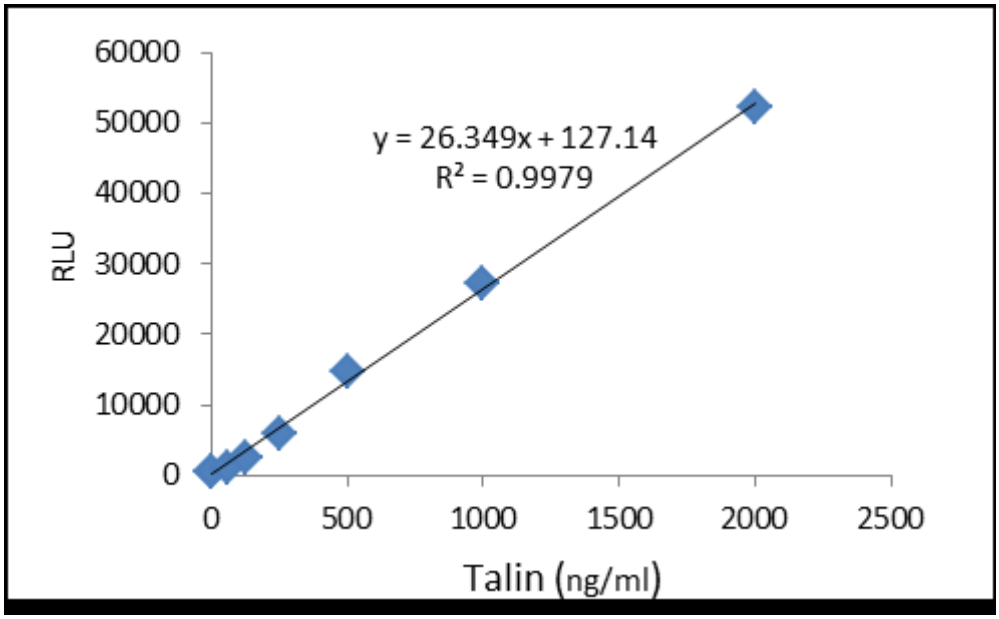

Figure 2: A typical calibration curve showing linear fit of the data with R^2 value of 0.9979.

LIMITATIONS AND PRECAUTIONS

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
- Do not mix reagents from different kits.
- Do not use previously generated calibration curve for data analysis. Generate a fresh calibration curve with each assay.
- The wash procedure is critical. Insufficient washing will result in poor precision and false absorbance readings.
- If the RLU values exceed the detection limit of the luminometer, the sample must be diluted and retested.

PERFORMANCE CHARACTERISTICS

Sensitivity

The assay range for this kit is from 0 to 2,000 ng/mL S-Talin with a limit of detection (LOD) of 30 ng/mL. The samples containing >2 µg/mL S-Talin (which results in RLU greater than that for 2000 ng/mL calibrator) should be diluted and retested.

Precision

Intra-Assay precision was determined by replicate determinations of S-Talin at three different concentrations (ng/mL) in serum samples in one assay. Intra-assay variability is shown below:

Sample	1	2	3
# Replicates	3	3	3
Mean	1975	1023	549
SD	123	122	56
CV %	6%	12%	10%

Inter-Assay precision was determined by replicate determinations of S-Talin at three different concentrations (ng/mL) in serum samples in 3 different assays. Inter-assay variability is shown below:

Sample	1	2	3
# Replicates	3	3	3
Mean	1967	1061	511
SD	10	54	54
CV %	1%	5%	11%

Recovery
Serum samples from healthy individuals with S-Talin concentration < 50 ng/mL were spiked with known amounts of recombinant S-Talin and assayed in duplicate. The mean recovery was 113%.

Specificity and Hook Effect
Studies on the specificty and hook effect were not performed for this kit.

Stability
The kit along with all the components is stable for at least six months when stored at 4℃. The lyophilized calibrator should be used within 4 hrs after reconstitution.

REFERENCES

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FOR RESEARCH USE ONLY

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