

Human/Mouse SMN Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE00027

Size: 96T

Sensitivity: 6.0 pg/mL

Range: 62.5-4000 pg/mL

Usage: For the quantitative detection of human/mouse SMN concentrations in cell lysate and tissue lysate.

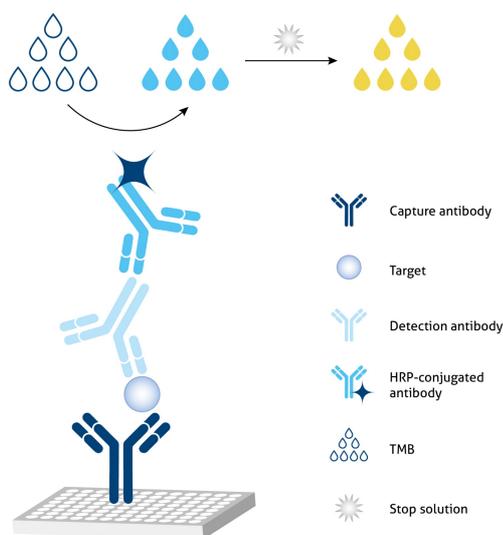
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1. Background

Survival Motor Neuron (SMN) protein required for efficient assembly of small nuclear ribonucleoprotein (snRNP) complexes is encoded by nearly identical telomeric and centromeric forms of SMN gene respectively. Both the SMN1 and SMN2 genes express SMN protein, however, the amount of functional full-length protein produced by SMN2 is much less than that produced by SMN1 due to the alternative splicing. The SMN gene is constitutively expressed in wide variety of tissues including brain, kidney, liver and spinal cord, while motor neurons are particularly vulnerable to reduced SMN protein levels. Deletion or mutational inactivation of the SMN1 gene causes spinal muscular atrophy (SMA), a lethal genetic disease characterized by loss of motor neurons in the spinal cord. The absence of SMN1 can be partially compensated for by SMN2 and the SMN2 expression level is associated with SMA severity. This kit is for the quantitative determination of SMN protein level in vivo.

2. Principle



Sandwich ELISA structure (HRP conjugated secondary antibody)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody also binds to the analyte. Horseradish peroxidase (HRP)-conjugated secondary antibody binds to the detection antibody. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

- 3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.
- 3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.
- 3.3 Plate washer: automated or manual.
- 3.4 Absorbent paper towels.
- 3.5 Glass or plastic tubes to prepare standard and sample dilutions.
- 3.6 Beakers and graduated cylinders.
- 3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

4. Kit Components and Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 8000 pg/bottle; lyophilized	2 bottles	
Detection antibody (100×) - 120 µL/vial*	1 vial	
HRP-conjugated antibody (100×) - 120 µL/vial*	1 vial	
Sample Diluent PT 3-af - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Extraction Reagent - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

- 5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.
- 5.2 Do not use the kit after the expiration date.
- 5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.
- 5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.
- 5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

6.1 Cell Lysate:

- 1) Collect cells and wash by centrifuging at 500 x g for 5 minutes before resuspension in pre-cooled PBS buffer. Perform this step three times.
- 2) Count cells and then discard the supernatant.
- 3) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing cell lysis.
- 4) Add 1 mL of Extraction reagent (containing protease inhibitor cocktail) Per 1×10^7 cells, Incubate cell suspension on ice for 30 minutes, use ultrasound to treat the samples.
- 5) Centrifuge cell lysate at 10,000 x g for 5 minutes at 4°C.
- 6) Measure the concentration of total protein in cell lysate using BCA assay. Where possible, keep samples on ice to avoid protein degradation.

6.2 Tissue Lysate:

- 1) Rinse tissue with PBS, cut into 1-2 mm pieces.
- 2) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing tissue lysis.
- 3) Add 1 mL of Extraction Reagent containing protease inhibitor cocktail per 100 mg tissue.
- 4) Homogenize the tissue completely using desired method on ice, Incubate on ice for 30 minutes, use ultrasound to break up the cells.
- 5) Centrifuge tissue homogenates at 10,000 x g for 5 minutes at 4°C. Collect the supernatant, assay immediately or aliquot and store at -20°C.
- 6) Measure the concentration of total protein in tissue homogenates using BCA assay.
- 7) Avoid protein degradation by performing all the above procedures on ice where possible.

7. Regent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

7.2 Detection Antibody (1X): Dilute 100X Detection Antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 μ L 100X Detection Antibody + 990 μ L Detection Diluent (Centrifuge the 100 X Detection Antibody solution for a few seconds prior to use).

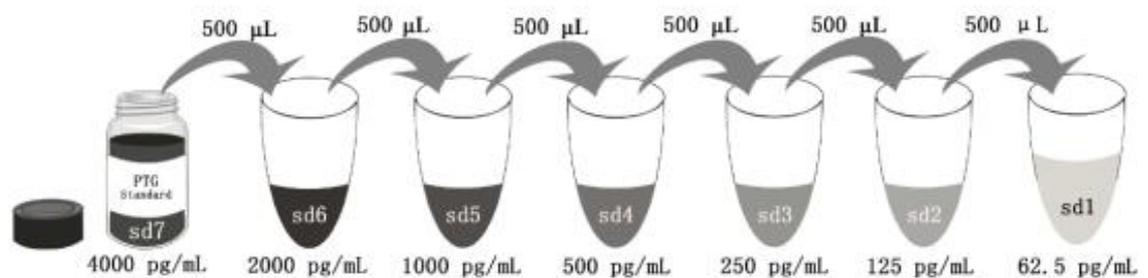
7.3 HRP-conjugated antibody (1X): Dilute 100X HRP-conjugated antibody 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 μ L 100X HRP-conjugated antibody + 990 μ L Detection Diluent (Centrifuge the 100X HRP-conjugated antibody solution for a few seconds prior to use).

7.4 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:100 is recommended for cell lysates; 1:2 or 1:4 or 1:8 is recommended for tissue lysates.

7.5 Standard Serial Dilution:

Add 2 mL Sample Diluent PT 3-af in protein standard.



Add # μ L of Standard diluted in the previous step	—	500 μ L					
# μ L of Sample Diluent PT 3-af	2000 μ L	500 μ L					
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody and HRP-conjugated antibody can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group, standard group and sample group, add 100 µL of each standard and sample to the appropriate wells. (Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, it is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 1 hour at 37°C.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL of 1X Detection Antibody solution (refer to Reagent Preparation 7.2) to each well. Seal plate with cover seal and incubate for 1 hour at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Add 100 µL of 1X HRP-conjugated antibody solution (refer to Reagent Preparation 7.3) to each well. Seal plate with cover seal and incubate the plate for 40 minutes at 37°C.

8.8 Repeat wash step in 8.4.

8.9 Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.10 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.11 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

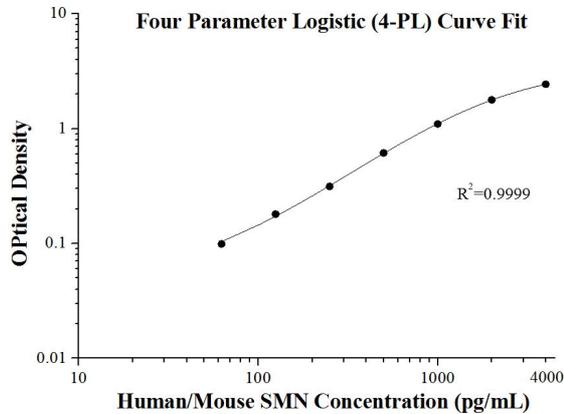
8.12 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve-fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 µL	60 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 µL	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 µL	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 µL	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 µL	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D	Average	Corrected
0	0.05 0.054	0.052	-
62.5	0.151 0.151	0.151	0.099
125	0.234 0.23	0.232	0.18
250	0.377 0.354	0.365	0.313
500	0.645 0.687	0.666	0.614
1000	1.134 1.167	1.1505	1.098
2000	1.818 1.84	1.829	1.777
4000	2.467 2.503	2.485	2.433

9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	20	1,037.6	63.2	6.1
2	20	1,678.6	71.1	4.2
3	20	2,465.5	98.0	4.0

Inter-assay Precision				
Sample	n	Mean (pg/mL)	SD	CV%
1	24	1,219.4	93.9	7.7
2	24	2,594.4	152.6	5.9
3	24	4,296	252.7	5.9

9.3 Recovery

The recovery of human/mouse SMN spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Matrix (# of samples)	Required Dilution	Spike Concentration (pg/mL)	Average% of Expected / Range (%)
Human cell lysate (n=4)	1:2	2,500	107 (106-108)
		1,250	92 (90-93)
		125	82 (71-94)
	1:4	2,500	89 (88-91)
		1,250	89 (83-90)
		125	98 (88-108)
Mouse skeletal muscle extract (n=4)	1:8	2,500	90 (90-91)
		1,250	88 (83-93)
		125	107 (93-121)
	1:16	2,500	107 (105-110)
		1,250	104 (95-112)
		125	112 (97-127)

9.4 Sample values

	Mouse muscle lysate	Mouse heart lysate	Mouse brain lysate	Mouse liver lysate	Mouse skin lysate	SHSY5Y cell lysate	Colo320 cell lysate
SMN /Total protein (pg/mg)	17.4	53.4	8.5	57.0	129.5	74	5524

9.5 Sensitivity

The minimum detectable dose of human/mouse SMN is 6.0 pg/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

9.6 Linearity

To assess the linearity of the assay, three samples were spiked with high concentrations of human/mouse SMN in various matrices and diluted with the appropriate **Sample Diluent PT 3-af** to produce samples with values within the dynamic range of the assay.

		Mouse muscle tissue	Human cell lysate
1:2	Average% of Expected	66	113
	Range (%)	62-71	101-124
1:4	Average% of Expected	79	111
	Range (%)	73-85	98-122
1:8	Average% of Expected	100	108
	Range (%)	100	100-116
1:16	Average% of Expected	114	98
	Range (%)	110-119	98-112

10. References

1. Lefebvre S, et al. Identification and characterization of a spinal muscular atrophy-determining gene. *Cell*. 80(1):155-65 (1995).
2. Gavrilov DK, et al. Differential SMN2 expression associated with SMA severity. *Nat Genet*. 20(3):230-1(1998).
3. Monani UR, et al. The human centromeric survival motor neuron gene (SMN2) rescues embryonic lethality in *Smn(-/-)* mice and results in a mouse with spinal muscular atrophy. *Hum Mol Genet*.9(3):333-9 (2000).
4. Burghes AH, et al. Spinal muscular atrophy: why do low levels of survival motor neuron protein make motor neurons sick ? *Nat Rev Neurosci*.10(8):597-609(2009).