⟨126⟩ SOMATROPIN BIOIDENTITY TESTS

Somatropin is a protein hormone that contains the same amino acid sequence as the human growth hormone produced by the pituitary gland. A robust and precise physicochemical chromatographic procedure is used in the assay to assign potency on a mass basis. Bioidentity is still required, and two procedure options are presented here: an in vivo bioassay procedure based on somatropin-induced weight gain in rats and a more precise rat cell line-based approach that measures production of ATP as a direct indicator of cell growth. [NOTE—The bioidentity test may be performed either on the somatropin bulk drug substance or on the drug product.]

PROCEDURE

- **IN VIVO BIOIDENTITY TEST**

Buffer solution: 0.1 M ammonium bicarbonate. Adjust with sodium hydroxide to a pH of 8.0.

Standard solutions: 10–100 µg/mL of USP Somatropin RS in *Buffer solution*

Sample solutions: 10–100 µg/mL of somatropin in *Buffer solution*. [NOTE—Do not agitate while mixing; swirl gently.]

Control: *Buffer solution*

Test animals: Select an appropriate number of only female or only male Sprague Dawley rats hypophysectomized at 25–30 days of age. After hypophysectomization, feed the rats rat chow and 5% dextrose water for at least 72 h. After 72 h, feed the rats rat chow and filtered and deionized water adjusted with 1 N hydrochloric acid to a pH of 3.0 ± 0.25. Weigh the rats when they are 37–44 days old, and retain only healthy rats. Reweigh the remaining rats 7 days later, and use only those rats that are in good health and have not gained or lost >10% of their body weight in the previous 7-day period.

Analysis: Randomly divide the rats into standard, test, and control groups, each group containing approximately 10 rats. Each day for 10 days inject subcutaneously 0.1 mL of the *Standard solutions*, *Sample solutions*, and *Control*, to the standard, test, and control groups, respectively. Record the body weight of each animal at the start of the test and at approximately 18 h after the tenth injection.

Calculations: Determine the change in body weight for each rat during the 10-day period, and compute the potency of the *Sample solution* relative to that of the *Standard solution* using appropriate statistical analysis. Calculate the mean potency in USP Somatropin Units/mg and, using appropriate statistical methods, calculate the width, *L*, of a 95% confidence interval for the estimated base 10 logarithm of the relative potency. If *L* is >0.40, repeat the test until the results from two or more tests, combined by appropriate statistical methods, produce an *L* of NMT 0.40, corresponding to confidence limits of 63%–158% of the calculated potency.

Acceptance criteria: NLT 2 USP Somatropin Units/mg when *L* is NMT 0.40

- **IN VITRO CELL-BASED BIOIDENTITY TEST**

Medium A: Fischer's medium[1] containing 10% heat-inactivated fetal bovine serum,[2] 10% heat-inactivated horse serum,[3] 0.075% sodium bicarbonate, and 0.05 mM 2-mercaptoethanol. Filter sterilize. [NOTE—Store for up to 2 weeks at 2°–8°.]

Medium B: Fischer's medium containing 1% horse serum, 0.075% sodium bicarbonate, and 0.05 mM 2-mercaptoethanol. Filter sterilize. [NOTE—Store for up to 2 weeks at 2°–8°.]

Phosphate buffered saline:[4] Calcium- and magnesium-free phosphate buffered saline containing 1.5 mM monobasic potassium phosphate, 155 mM sodium chloride, and 3 mM dibasic sodium phosphate, pH 7.2–7.4

Cell culture preparation: Prepare cell suspension cultures of Nb2-11[5] cells in *Medium A* in a humidified incubator at 37° and containing 5% carbon dioxide. Cells should be passaged twice per week and reseeded at a density of 1×10^5 cells/mL for 2 days, 2×10^4 cells/mL for 3 days, and 1×10^4 cells/mL for 4 days in *Medium A*. [NOTE—Seeding densities may need adaptation when analysts qualify new lots of fetal bovine serum and horse serum.] On the day of an assay, cells are harvested from flasks and are pelleted by centrifugation for 7 min at about 218 × g. The supernatant is discarded, and the cells are washed twice with *Phosphate buffered saline* followed by centrifugation. The cells are resuspended in *Medium B* and are counted, and the cell concentration is adjusted to 1×10^5 cells/mL with *Medium B*. Except for the wells of the first column, transfer 50 µL of cell suspension into each well of a 96-well black tissue culture plate with a clear bottom.[6] Pipet 50 µL of *Medium B* into each well of the first column of the plate. [NOTE—Cover the plate, and incubate at 37° for up to 1 h while preparing the *Standard solutions* and *Test solutions*.]

Standard solutions: Reconstitute USP Somatropin RS in 1 mL of *Phosphate buffered saline*. From the reconstituted USP Somatropin RS, the *Standard solutions* and the *Positive control solution* are prepared for use in the *Procedure*. For the *Standard solutions*, the reconstituted USP Somatropin RS is further diluted with *Medium B* to a concentration of 2.0 ng/mL. [NOTE—Use polypropylene test tubes to make dilutions. For each plate approximately 1 mL of this solution is needed. Do not use single step dilutions of more than 1:100 or smaller transfer volumes than 40 µL.]

Test solutions: For each test solution two results must be obtained by two independent preparations of somatropin. Reconstitute two independent preparations of somatropin in a suitable diluent (e.g., *Phosphate buffered saline* or *Water for Injection*) to a concentration of NMT 5 mg/mL. Prepare *Test solutions* by diluting this material further with *Medium B* to a concentration of 2.0 ng/mL. [NOTE—Use polypropylene test tubes to make dilutions. For each plate approximately 1 mL of this solution is needed. Do not use single step dilutions of more than 1:100 or smaller transfer volumes than 40 µL.] One preparation is *Test solution A*, and the other preparation is *Test solution B*.

Positive control solution: Starting with the reconstituted stock preparation of USP Somatropin RS described in *Standard solutions*, dilute with *Medium B* to a concentration of 20 ng/mL. [NOTE—Use polypropylene test tubes to make dilutions. Do not use single step dilutions of more than 1:100 or smaller transfer volumes than 40 µL.]

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[1] Quality Biological, catalog #112-032-101, or equivalent.
[2] Gibco, catalog #10082-147, or equivalent.
[3] Gibco, catalog #26050-088, or equivalent.
[4] Amimed catalog, #8-05F29I; Gibco catalog, #20012-019, or equivalent.
[5] HPA Culture Collections or Sigma-Aldrich, catalog #97041101.
[6] Costar, catalog #3904, or equivalent.

Procedure: Dispense 100 µL of *Medium B* into each well of a U-bottom, clear 96-well plate,[7] except those wells in column 12 (wells A12–H12 = positive control) and wells A2–A10. [NOTE—For each black plate seeded with cells as described in *Cell culture preparation,* one separate U-bottom plate is used for preparation of the *Standard solutions* and *Test solutions* dilutions.] Next, dispense 200 µL of 2.0-ng/mL *Standard solutions* into wells A3, A6, and A9 of the dilution plate. Dispense 200 µL of 2.0-ng/mL *Test solution A* into wells A2, A5, and A8 of the dilution plate. Dispense 200 µL of 2.0-ng/mL *Test solution B* into wells A4, A7, and A10 of the dilution plate. Dispense 100 µL of 20-ng/mL *Positive control solution* into the last plate column (wells A12–H12) of the dilution plate. Using a 12-channel pipet, perform serial two-fold dilutions on the plate by aspirating 100 µL from the first row (A2–A10), transfer to the second row, and mix three times. Then aspirate 100 µL from the second row, transfer to the third row, and mix three times, etc. Repeat this procedure across the whole plate up to row H. Discard the 100 µL aspirated from the last row. [NOTE—The wells of column 11 (wells A11–H11) are negative controls containing 100 µL of *Medium B*; the wells of column 1 (wells A1–H1) are blanks.]

Starting with the lowest concentration and using a 12-channel pipet, transfer 50 µL of each solution in the dilution plate to the respective well of the black plate containing the cells. [NOTE—During the transfer, immerse the pipet tips into the cell solution. This transfer yields an additional 1:2 dilution of somatropin, or a final concentration of 1.0 ng/mL for the highest dose added to the cells and 10 ng/mL for the *Positive control solution*. A plate layout is shown in *Table 1*.] Following all transfers, gently agitate the black plate for 30 s, and then incubate it in a humidified 37° incubator containing 5% carbon dioxide for 30 ± 2 h. Next, add 100 µL of reconstituted luminescent substrate solution[8] to all wells. Incubate the plates for 15 min at room temperature while gently agitating the plates on an appropriate plate shaker, protected from light. Incubate the plates for an additional 15 min at room temperature without shaking, protected from light. Read the luminescence from the plate wells in a suitable plate reader with a luminescence detection mode.

Table 1. Schematic Representation of the Final Assay Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	A,#1	St,#1	B,#1	A,#1	St,#1	B,#1	A,#1	St,#1	B,#1	NEG	POS
B	BL	A,#2	St,#2	B,#2	A,#2	St,#2	B,#2	A,#2	St,#2	B,#2	NEG	POS
C	BL	A,#3	St,#3	B,#3	A,#3	St,#3	B,#3	A,#3	St,#3	B,#3	NEG	POS
D	BL	A,#4	St,#4	B,#4	A,#4	St,#4	B,#4	A,#4	St,#4	B,#4	NEG	POS
E	BL	A,#5	St,#5	B,#5	A,#5	St,#5	B,#5	A,#5	St,#5	B,#5	NEG	POS
F	BL	A,#6	St,#6	B,#6	A,#6	St,#6	B,#6	A,#6	St,#6	B,#6	NEG	POS
G	BL	A,#7	St,#7	B,#7	A,#7	St,#7	B,#7	A,#7	St,#7	B,#7	NEG	POS
H	BL	A,#8	St,#8	B,#8	A,#8	St,#8	B,#8	A,#8	St,#8	B,#8	NEG	POS

LEGEND:
BL = Blank: no somatropin, no cells.
A = Dilution series of *Test solution A* (with 1.0 ng/mL as starting concentration).
St = Dilution series of *Standard solutions* (with 1.0 ng/mL as starting concentration).
B = Dilution series of *Test solution B* (with 1.0 ng/mL as starting concentration).
NEG = Negative control: no somatropin, but cells are present.
POS = *Positive control solution:* maximum somatropin concentration (10 ng/mL).

Calculations: A minimum of two independent *Test solutions* preparations must be used for each test sample. The *Test solutions* and *Standard solutions* are normalized by protein content before calculation of the relative potency. [NOTE—1 mg of anhydrous somatropin is equivalent to 3.0 USP Somatropin Units.] Technical outliers (but NMT four per curve) are omitted, and then the same number of *Standard solutions* and *Test solutions* dose responses, including the 50% response (EC_{50}) of the standard per test sample within this range, are used to calculate the relative potency of each somatropin sample using statistical methods for parallel-line analysis. For each of the individual *Test solutions* compared to the *Standard solutions*, the statistical tests for linearity, slope, and parallelism must pass at the 95% level. The confidence limit for each calculated relative potency must be within 75%–133%. The relative potency of each of the *Test solutions* must be within the validated assay range (50%–200% relative potency) of the *Standard solutions*. The unweighted mean log relative potency is calculated from valid individual samples, and the potency is then calculated in USP Units/mg relative to the USP Somatropin RS.

System suitability criteria: The signal-to-noise ratio of the mean chemiluminescence signal of the *Positive control solution* to the mean chemiluminescence signal of the negative control wells must be NLT 3. NMT four technical outliers may be omitted per standard curve. Any plate that fails one or more of these criteria is rejected and must be repeated.

Acceptance criteria: NLT 2 USP Somatropin Units/mg

• **USP REFERENCE STANDARDS** ⟨**11**⟩
USP Somatropin RS

[7] Greiner #650 160 plate or equivalent.
[8] CellTiter-Glo Luminescent Kit, e.g., Promega #G7573, or equivalent.