

Human PTH (1-34)

**Immunoradiometric Assay (IRMA)
(Coated Tube-Technology)
For the quantitative determination
of human PTH (1-34)
For *in vitro* Research Use Only**

Scantibodies

(Part Number: 3KG034)

Store at 2 – 8° C

INTENDED USE

This kit is intended for research use only in the quantitative determination of immunoreactive Human PTH (1-34) in blood samples, not for use in diagnostic procedures.

PHYSIOLOGY

The cyclase activating PTH peptide (1-84) is secreted by parathyroid glands under the regulation of the extracellular concentration of ionized calcium, vitamin D and magnesium (1, 2, 3). PTH acts with respect to calcium on the kidneys and the skeleton. PTH binds to the PTH/PTHrP receptors, which stimulate adenylate-cyclase to produce cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP) (4-7). Primary and secondary hyperparathyroidism, kidney insufficiency, malabsorption-syndrome and pseudo-hypoparathyroidism result in elevated concentrations of PTH (8, 9). Decreased concentrations of PTH coincide with high doses of vitamin-D, milk-alkali-syndrome, and hypercalcemia of malignancy. PTH concentration is also decreased with absorptive hypercalciuria and hypoparathyroidism (10-12).

The biological activity of PTH resides in the first two amino acids of the N-terminal portion of the molecule (13-16). PTH is metabolized either intra glandular or in the peripheral organs into fragments. Circulation PTH is immunologically heterogenous. PTH (1-34), a PTH fragment that retains the intact N-terminal region, is reported to elicit the full spectrum of bone-relevant activities that is characteristic to the whole PTH (1-84) hormone (17-20). Due to its short peptide chain, PTH (1-34) is undetectable by either the major iPTH assays or the whole PTH assays available in the market. Several pharmaceutical companies are marketing recombinant or synthetic PTH (1-84) for treatment of osteoporosis. Comparison of the biological activities of PTH (1-84) and PTH (1-34) in receptor activation, stimulation of osteoblasts and bone turn-over may provide information for receptor signal transduction, bone metabolism and pathological development and potential treatment of chronic kidney disease and osteoporosis.

PRINCIPLE OF PROCEDURE

Scantibodies Human PTH (1-34) is an immunoradiometric assay (IRMA) utilizing a polyclonal

PTH antibody with a tendency to bind in the N-Terminal region of PTH (1-34) (Label Antibody), and a polyclonal PTH antibody with a tendency to bind in the C-Terminal region of PTH (1-34) (Capture Antibody). The PTH (1-84) is extracted from the specimens by sample pre-treatment in PTH (1-84) extraction tubes. The use of these antibodies guarantees that this assay detects only the biologically active PTH (1-34) which contains the first two amino acids of the N-terminal portion of the molecule. The Label Antibody is labeled with ¹²⁵I. The Capture Antibody is fixed to the tubes. The PTH (1-34) in patient samples is bound both to the tubes and the Label Antibody. After incubation free ¹²⁵I antibodies and bound ¹²⁵I antibody fractions are separated by discarding the supernatant. Simple wash steps reduce the non-specific binding (NSB) to a minimum for increased precision at the low end of the calibration curve. The concentration of PTH (1-34) is directly proportional to the radioactivity bound to the tubes after separation. The concentration of PTH (1-34) in unknown patient samples and controls is determined by interpolation using a calibration curve.

REAGENTS

The Scantibodies Human PTH (1-34) IRMA Kit contains sufficient reagents for 100 single determinations. The kit is stable at 2 - 8° C until the stated expiration date.

Human PTH (1-34) Standards

One set of standards consists of seven vials containing lyophilized human serum with nominal PTH concentrations. The lyophilized standards are prepared in stabilized human serum containing 0.1% (w/v) sodium azide. The PTH concentrations are declared on the vial label.

Human PTH (1-34) Controls

One set of controls consists of two vials containing PTH in lyophilized human serum with 0.1% (w/v) sodium azide. The concentration ranges of PTH are declared on the vial labels.

Goat Anti-N-Terminal PTH ¹²⁵I Ab

One set of tracer consists of two bottles of Goat Anti-N-Terminal PTH ¹²⁵I Ab. Each bottle contains polyclonal goat anti N-Terminal PTH which is labeled with ¹²⁵I and dissolved in 5 ml phosphate buffered saline with 0.1% (w/v) sodium azide and protein stabilizers. The maximum radioactivity in a bottle is <370 kBq (<10 μCi). This kit contains ¹²⁵I (half life: 60 Days), emitting ionizing X (28 keV) and Gamma γ (35.5 keV) radiations.

Human PTH Ab Coated Tubes

Two packages of 50 tubes each plus desiccant. The tubes are coated with polyclonal goat anti-PTH. The desiccant contains silica.

Human PTH (1-84) Extraction Tubes

One package of 50 tubes plus desiccant. The tubes are provided for patient sample pre-treatment. The desiccant contains silica.

Wash Concentrate

One bottle contains 30 ml of a 30 fold concentrate of phosphate buffered saline with 1.5% (w/v) sodium azide and detergent.

PREPARATION AND STORAGE OF REAGENTS

Human PTH (1-34) Standards

The Scantibodies Human PTH (1-34) IRMA kit contains the PTH standards prepared analytically on a mass basis from purified synthetic PTH peptide. These standards are further evaluated against "primary standards" which are stored at -70° C to maintain calibration. Reconstitute the zero standard with 5 ml of distilled or deionized water. Reconstitute the remaining standards with 2 ml of distilled or deionized water. After addition of the water, mix each vial thoroughly but gently. Use the reconstituted standards within 1 hour. Store the unused portion of the standards below -20° C until the stated expiration date. Do not store the standards at room temperature for more than one hour at any given time. Do not thaw any standard vial more than two times. Do not use standards that exhibit precipitation or unusual color.

Human PTH (1-34) Controls

Reconstitute the vials of controls with 2 ml of distilled or deionized water. After addition of the water, mix each vial thoroughly but gently. Use the reconstituted controls within 1 hour. Store the unused portion of the controls below -20° C until the stated expiration date. Do not store the controls at room temperature for more than one hour at any given time. Do not thaw any control vial more than two times. Do not use controls that exhibit precipitation or unusual color.

Goat Anti N-Terminal PTH ¹²⁵I Ab

The tracer is ready to use. Store the tracer at 2 - 8° C until the stated expiration date. Do not use tracer that shows precipitation or unusual color.

Human PTH Antibody Coated Tubes

The antibody coated tubes are ready to use. Store the tubes at 2 - 8° C until the stated expiration date. Allow the tubes to equilibrate to room temperature prior to use. Reseal the package immediately after removing the required number of tubes.

Human PTH (1-84) Extraction Tubes

The PTH (1-84) Extraction tubes are ready to use. Store the tubes at 2 - 8° C until the stated expiration date. Allow the tubes to equilibrate to room temperature prior to use. Reseal the package immediately after removing the

required number of tubes.

Wash Concentrate

Dilute and thoroughly mix the 30 ml of wash concentrate with 870 ml of distilled or deionized water (1:30). Store the diluted wash solution at room temperature (18 - 25° C) until the stated expiration date. Do not use wash solution that shows precipitation.

WARNINGS AND PRECAUTIONS FOR USERS

Use of the Assay

The reagents are for in vitro research use only, not for diagnostic procedures.

Human Serum Caution

The human serum in this kit has been prepared from human donors and it has been tested by FDA approved immunoassays and found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Anti HIV I/II and Anti HCV. However, it is recommended to consider the standards and controls as a potential biohazard and handle them with the same precautions as applied to any untested patient sample.

Radioactivity Warning

This radioactive material may be received, acquired, possessed, or used only by physicians, clinical laboratories, or hospitals and only for *in vitro* or laboratory tests not involving internal or external administration of the material, or the radiation there from, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations and a general license of the U.S. Nuclear Regulatory Commission or of a state with which the Commission has entered into an agreement for the exercise of regulatory authority.

All radioactive materials must be disposed of according to the regulations (regulations differ from country to country) and guidelines of the agencies with jurisdiction over the laboratory. Do not eat, drink, smoke or apply cosmetics in areas where radioactive materials are used. Storage of radioactive materials should be limited to specifically designated and appropriately secured areas. Access to radioactive materials should be limited to authorized and trained personnel only. Do not pipette radioactive solutions by mouth. Avoid direct contact with radioactive materials by using protective articles such as lab coats and disposable gloves. Radioactive materials must be stored in designated areas in their original containers or in containers providing equivalent radiation protection. A record of disposal of all radioactive materials must be kept. Immediately remove spilled solutions and decontaminate contaminated devices. Check laboratory equipment and glassware regularly to detect contamination with radioisotopes.

Sodium Azide (NaN₃) Warning

Some reagents in the Scantibodies Human PTH (1-34) IRMA Kit contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush the drain with a large

volume of water to prevent azide build-up. Avoid direct contact with skin and mucous membranes.

SAMPLE PREPARATION AND STORAGE

Specimen Collection

The determination of Human PTH (1-34) must be made on EDTA-plasma. Five hundred microliters (500 μ L) of plasma are required to assay one sample in duplicate for Human PTH (1-34) values. To obtain plasma, collect blood by venipuncture into EDTA tube. Centrifuge the sample at 2 - 8° C and separate the plasma from the cells. Plasma should be stored at -20° C or lower. Avoid repeated freezing and thawing of plasma. Do not use patient samples which have been frozen and thawed more than two times.

Dilution of Patient Samples

Dilute patient samples with PTH concentrations greater than the highest standard with Scantibodies PTH (1-34) Zero Standard before assay. The dilution factor is applied to the diluted sample assay result in order to determine the PTH (1-34) concentration in the undiluted sample.

Quality Control

Two levels of controls are provided with each assay kit. The values assigned to these controls are printed on the container label. The control value should fall within the specified range when tested in the same manner as the unknowns. Controls should be included in each assay. If the control values do not meet the established range, the assay may be invalid and should be repeated.

ASSAY PROCEDURE

Materials Provided

The Scantibodies Human PTH (1-34) IRMA Kit (Part No. 3KG034) is supplied with the following:

Description	Number
Human PTH (1-34) Standards Part Nos. 3CA037, 3CB037, 3CC037, 3CD037, 3CE037, 3CF037, 3CG037	7 vials
Human PTH (1-34) Controls Part Nos. 3CA038, 3CB038	2 vials
Human PTH (1-84) Extraction Tubes Part No. 3KT026	1 package of 50 tubes
Human PTH Ab Coated Tubes Part No. 3KT027	2 packages of 50 tubes
Goat Anti N-Terminal PTH ¹²⁵ I Ab Part No. 3KL022	2 vials
Wash Concentrate Part No. 3KW001	1 bottle
Human PTH (1-34) IRMA Directional Insert Part No. 7KI071	1 insert

Materials and Equipment Required but Not Provided:

Distilled or deionized water
Round-bottomed polypropylene or polystyrene test tubes (12 x 55, 12 x 75, 12 x 70 mm or equivalent)
Pipetter with disposable tips: 0.2 ml
Repeating dispenser: 0.1 ml

Wash station
Vortex mixer
Gamma counter calibrated to detect ¹²⁵I

Reconstitution of Standards and Controls

Reconstitute the Scantibodies PTH standards and controls as described in the directional insert.

Patient Sample Extraction

PTH (1-84) is extracted from the patient sample in a PTH (1-84) Extraction tube. Pipette 0.5 ml patient samples into the PTH (1-84) Extraction tubes (3KT026), gently vortex, and incubate at room temperature (18 - 25° C) for 4 hours on an orbital shaker at 170 rpm. After extraction the processed patient samples will be tested in the following steps for PTH (1-34) determinations.

No extraction is to be performed for the standards and controls. However, the reconstituted standards and controls are also incubated **in the original vials** together with the patient samples at room temperature (18 - 25° C) for 4 hours on an orbital shaker at 170 rpm

Assay Set up

Prepare the following groups of tubes and place them in a test tube rack during the 4-hour sample extraction. The samples, standards and controls are tested in duplicates.

Two total count (TC) tubes (optional for QC) (use polypropylene tubes).

Two Bo tubes (NSB) (3KT027) provided with kit.

Two PTH antibody-coated tubes (3KT027) for each standard concentration - provided with kit.

Two PTH antibody-coated tubes (3KT027) for each control concentration - provided with kit.

Two PTH antibody-coated tubes (3KT027) for each patient sample - provided with kit.

Pipetting after the Sample Extraction

- Pipette 0.2 mL of standards, controls and patient samples [post-extraction in the PTH (1-34) extraction tubes 3KT026] into the PTH antibody-coated tubes (3KT027).
- Pipette 0.1 ml of Goat Anti N-Terminal PTH ¹²⁵I Ab (3KL022) into each tube.
- Gently vortex all tubes.
- Seal the tubes.
- Incubate the standards, controls and samples for 18 - 24 hours at room temperature (18 - 25° C).
- Aspirate the supernatant from each tube except for the TC tubes. Wash the tubes 3 times with 2 ml of diluted wash solution. After each addition of diluted wash solution aspirate all of the wash solution.
- Count each tube for at least 1 minute in a gamma counter calibrated to detect ¹²⁵I. The total count tube should contain approximately 300,000 CPM (assuming the counter has an efficiency of 70% - 80%) when freshly iodinated tracer is used. The total activity of the tracer decreases according to the half-life of ¹²⁵I.

PIPETTING GUIDE

Additive To Tube	TC Tubes	Bo Tubes	Standard Tubes	Control Tubes	Sample Tubes
Standard	-	200 μ l	200 μ l		-
Control	-	-	-	200 μ l	
Sample	-	-	-	-	200 μ l
PTH Tracer	100 μ l	100 μ l	100 μ l	100 μ l	100 μ l
Gently vortex all tubes, except for the TC tubes. Incubate tubes for 18 - 24 hours at room temperature (18 - 25° C).					
Aspirate the supernatant from all of the tubes except the TC tubes. Wash all tubes except the TC tubes by adding 2 ml of diluted wash solution and aspirating the wash solution. Repeat this wash step two more times for a total of three times.					
Count each tube for at least 1 minute in a gamma counter.					

PROCEDURAL COMMENTS

Known Interferences:

Grossly hemolyzed or lipemic samples.

Samples from patients receiving radioisotopes for diagnostic or therapeutic purposes.

Contamination of the sample or assay tube with 125 I or other radioisotopes.

Samples containing up to 250 mg/dl triglyceride, 15 mg/dl hemoglobin and 15 mg/dl bilirubin do not exhibit any effect on the assay within the medical decision point for this assay.

Reagents from different lot numbers must not be interchanged.

The patient sample or standard and tracer should be pipetted carefully into the bottom one-fourth of the assay tube. This is to avoid losing liquid on the surface of the tube as the liquid runs down the tube.

The washing step is an important step in the assay procedure. Accurate dispensing of the wash solution and complete aspiration of the tube contents is essential to achieving assay sensitivity, low background and assay precision.

It is recommended that standards, controls, and patient samples be assayed in duplicate. The average counts per minute of each duplicate should then be used for data reduction and the calculation of results.

Standards and controls must be frozen immediately after use and may only be thawed and reused a maximum of two times provided acceptable control results are obtained.

Avoid sample to sample contamination by using a new pipette tip for each sample.

Proper reconstitution of the standards and controls in the assay is critical as any under or over reconstitution

may result in a faulty result including poor recovery and precision.

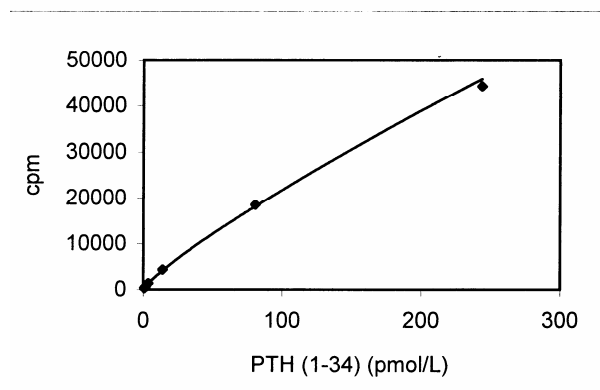
Studies have shown that samples stored at 2 - 8° C or room temperature for any significant amount of time may degrade.

CALCULATION OF RESULTS

1. Calculate the average CPM for each double determination.
2. Subtract the average CPM of the zero standard tubes from the CPM's from all other tubes in order to obtain the corrected CPM for each tube.
3. Corrected CPM = average CPM of duplicate samples - average CPM of duplicate zero standards.
4. Draw the calibration curve by plotting the average corrected CPM from each duplicate standard level (ordinate) against the respective concentration declared on the standard vial (absolute) using log-log graph paper. Obtain sample concentrations by interpolation of average sample CPM on the calibration curve.
5. If samples were run with dilution, multiply the diluted sample assay results from the curve by the appropriate dilution factors to obtain the undiluted sample assay results.

*Conversion of unit of measure: pmol/L x 4.118 \rightarrow pg/ml

REPRESENTATIVE CALIBRATION CURVE



Automated data reduction can also be used to construct the Scantibodies Human PTH (1-34) IRMA calibration curve. To program automated data reduction systems or to adapt an existing program, consult the data processor manufacturer or the programmer.

LIMITATIONS OF THE PROCEDURE

The assay procedure must be followed exactly; careful technique must be used to obtain valid results. Any modification of the assay procedure is likely to alter the results.

Grossly hemolyzed, lipemic or iceteric samples are likely

to give non valid results.

The highest concentration of PTH measurable without sample dilution is the concentration of the highest standard. The lowest level measurable is approximately 0.15 fmol/ml.

EXPECTED VALUES

The expected values were determined with 60 samples from apparently healthy individuals based on 95% confidence intervals. The PTH (1-34) values determined were ≤ 0.6 pmol/L which approximates or below the functional sensitivity. Therefore, this assay is not designed for accurate measurement on samples in the normal range. It is recommended that each laboratory establish its own range of normal values. The values given are only indicative and may vary from other published data.

PERFORMANCE CHARACTERISTICS

Accuracy, Recovery

Different plasma samples with low concentrations of PTH were spiked with specified amounts of PTH (1-34). The % recovery was determined following assay of the spiked samples.

PTH (1-34) Accuracy, Recoveries					
Samples	Sample value (pmol/L)	Added PTH (1-34) (pmol/L)	Expected Values (pmol/L)	Measured Values (pmol/L)	Recoveries (%)
1	0.6	3.78	4.38	4.53	103.4
	0.6	46.24	46.84	44.63	95.3
	0.6	92.92	93.52	87.84	93.9
2	0.23	3.94	4.17	3.65	87.5
	0.23	41.94	42.17	41.57	98.6
	0.76	85.82	86.58	85.22	98.4
3	1.03	3.90	4.93	4.99	101.3
	1.03	45.28	46.31	40.7	87.9
	1.03	89.18	90.21	88.18	97.8
Mean					96.0

Accuracy, Dilution

Different plasma samples with high concentrations of PTH (1-34) were diluted in a sample with low concentrations of PTH (1-34). The % recovery was determined following assay of the diluted samples.

PTH (1-34) Accuracy, Dilution				
Samples	Dilutions	Expected Values (pmol/L)	Measured Values (pmol/L)	Recoveries (%)
1	Neat		7.65	
	1:2	3.83	3.65	95.4
	1:4	1.91	2.1	109.8
2	Neat		85.12	

PTH (1-34) Accuracy, Dilution				
Samples	Dilutions	Expected Values (pmol/L)	Measured Values (pmol/L)	Recoveries (%)
	1:2	42.56	42.89	100.8
	1:4	21.28	20.56	96.6
	1:8	10.64	9.80	92.1
	1:16	5.32	4.60	86.5
	1:32	2.66	2.46	92.5
3	Neat		176.64	
	1:2	85.82	88.89	103.6
	1:4	42.91	43.37	101.1
	1:8	21.46	21.23	99.0
	1:16	10.73	9.96	92.8
	1:32	5.36	4.78	89.1
	1:64	2.68	2.61	97.3

High Dose Hook Response

The high dose hook response of the Scantibodies Human PTH (1-34) IRMA assay was determined using human plasma samples with spiked synthetic PTH (1-34) peptide as the unknowns. At approximate 5,000 pmol/L (approximately 20 times of the highest standard), the signal response (measured in CPM) in the PTH (1-34) spiked sample was more than 50% higher than that of the highest standard.

Precision

Inter-assay coefficient of variation was evaluated by performing 20 different assays on two PTH (1-34) spiked EDTA plasma samples.

PTH (1-34) Precision, Inter-assay				
Samples	Kit Batch	Mean Values (pmol/L)	SD (pmol/L)	CV (%)
1	E1	10.31	0.55	5.3
	E2	10.08	0.69	6.9
	E3	10.20	0.70	6.9
2	E1	82.23	3.44	4.2
	E2	85.44	5.00	5.9
	E3	82.13	4.87	5.9

Intra-assay coefficient of variation was evaluated by performing 20 replicate determinations on three PTH (1-34) spiked EDTA plasma samples in the same assay.

PTH (1-34) Precision, Intra-assay				
Samples	Kit Batch	Mean Values (pmol/L)	SD (pmol/L)	CV (%)
1	E1	9.34	0.23	2.5
	E2	8.30	0.29	3.5
	E3	8.12	0.29	3.6
2	E1	93.18	1.68	1.8
	E2	91.54	3.32	3.6
	E3	97.66	2.51	2.6

Sensitivity

The detection limit of the assay is defined as the lowest measurable value distinguishable from zero. This sensitivity was determined by assaying the zero standard 20 times in the same assay. The detection limits determined is approximately 0.15 pmol/L for Scantibodies Human PTH (1-34) IRMA at two standard deviation above the geometric mean of PTH zero standard. The functional sensitivity is defined as being the measured concentration by imprecision profile for a CV equal to 20%. It has been assessed as being 0.5 pmol/L.

Specificity

The following table presents the specificity of the Scantibodies Human PTH (1-34) IRMA.



























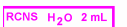
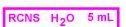
PTH (1-34) Cross-reactivity			
Human PTH Peptides	Peptide Conc. (pmol/L)	Measured Conc. (pmol/L)	Cross Reactivity (%)
PTH (1-84)	106.1	5.32	5.0
	53.05	3.18	6.0
	26.53	1.57	5.9
	10.61	0.77	7.3
PTH (7-84)	1,138.69	0.36	0.03
	113.87	0.34	0.3
	56.94	0.3	0.5
	11.39	0.24	2.1
PTH (39-84)	242.90	0.27	0.1
	121.45	0.3	0.2
	60.73	0.29	0.5
	24.29	0.26	1.1







CHEMICAL CHARACTERIZATION AND HAZARDOUS INGREDIENTS

Chemical Characterization:	1) Antibodies coated on to polystyrene Tubes
	2) Radioactive Isotope containing Iodine-125 with radioactivity <10 µCi and Sodium Azide @ 0.1%.
	3) Standards & Controls – Human Serum containing Sodium Azide @ 0.1%
	4) Wash Concentrate containing sodium azide @ 1.5%.
Hazardous Ingredients:	Radioactive Isotope (Iodine-125) @ <10 µCi/Vial (<370 kBq) CAS Number: 7553-56-2 Symbols: Harmful Xn R-phrases: R22, R52/53 S-phrases: S28, S45, S53, S60, S61

Hazardous Ingredients:	Sodium Azide @ 0.1% CAS Number: 026628-22-8 Symbols: N/A R-phrases: N/A S-phrases: N/A
	Sodium Azide @ 1.5% CAS Number: 026628-22-8 Symbols: Very Toxic T+; N R-phrases: R28, R32, R50/53 S-phrases: S28, S45, S53, S60, S61

SYMBOLS USED

Symbol	Used for	Symbol	Used for
	Do Not Reuse		Use By YYYY-MM-DD or YYYY-MM
	Batch Code		Serial Number
	Date of Manufacture		Sterile
	Sterilized Using Aseptic Processing Technique		Catalog Number
	Caution, Consult Accompanying Documents		Biological Risks
	Manufacturer		Authorized Representative in the European Community
	Contains Sufficient for < n > Tests		For IVD Performance Evaluation Only
	<i>In vitro</i> Diagnostic Medical Device		Upper Limit of Temperature
	Lower Limit of Temperature		Temperature Limitation
	Consult Instructions for Use		Positive Control
	Control		Negative Control
	Antibody Coated Beads		Antibody Coated Tubes
	Radioactive Iodine Tracer		Wash Solution
	Reconstituted with 2 mL		Reconstituted with 5 mL Water

Symbol	Used for	Symbol	Used for
	Water		
	European Conformity Mark		Radioactive
	Toxic		Harmful
	Corrosive		Oxidizing

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