BioVendor Group

ENG

Instructions for Use:
hPTH-ELISA

Catalogue number:
RIS003R

For research use only!

BioVendor R&D

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CONTENT

HISTORY OF CHANGES

Previous version	Current version
ENG.006.A	ENG.007.A
changing the structure of the document (order of chapters)	
	Removal of the column relative to colour code
Chapter **4** Conjugate: HRP labelled antiPTH (monoclonal antibodies) in TRIS-maleate buffer with bovine serum albumin, thymol and sheep serum (11ml) Chromogenic Solution (TMB: Tetramethylbenzydine) (25 ml) Stopping reagent HCl 1N (25ml)	Chapter **4** – Conjugate: HRP labelled antiPTH (monoclonal antibodies) in TRIS-maleate buffer with bovine serum albumin, thymol and sheep serum (13 ml) – Chromogenic Solution (TMB: Tetramethylbenzydine) (13 ml) – Stopping reagent H_2SO_4 0.2 M (13ml)
	Chapter **11** changed values in the table
Chapter **12.1** Twenty zero calibrators were assayed along with a calibrators. The detection limit, defined as the apparent c two standard deviations above the average OD at zero 2 pg/ml.	Chapter **12.1** Twenty zero calibrators were assayed along with a set of other calibrators. The detection limit, defined as the apparent concentration two standard deviations above the average OD at zero binding, was 0.8 pg/ml.
Chapter **12.2** Cross-reactive hormones or fragments were added to the zero calibrator, a high value calibrator (900 pg/ml) and a low value calibrator (100 pg/ml). The apparent PTH response was measured	Chapter **12.2** Cross-reactive hormones or fragments were added to the zero calibrator and two serum samples with different hPTH concentrations. The apparent PTH response was measured
Chaptres **12.3** and **12.4**.: changed values in the tables	

1. INTENDED USE

Immunoenzymetric assay for the *in vitro* quantitative measurement of human Intact Parathyroid Hormone (PTH) in serum and plasma.

2. CLINICAL BACKGROUND

2.1 Biological activities

Human parathyroid hormone (hPTH) is a major physiological regulator of phosphocalcic metabolism. hPTH increases serum calcium concentrations by its actions on kidney (enhancing tubular Ca^{++} reabsorption and phosphate excretion) and bone (stimulating osteoclastic activity and bone resorption). It indirectly affects intestinal absorption of Ca^{++} by stimulating renal 1α-hydroxylation of 25 hydroxyvitamin D. The release of PTH is controlled in a negative feedback loop by the serum concentration of Ca^{++}.
PTH is synthesized in the chief cells of the parathyroid glands and secreted as an 84 amino acid molecule called "intact PTH", which is the main bioactive product. This molecule is degraded by proteolytic cleavage between amino acids 33-37 at peripheral sites to form biologically active

amino- terminal fragments and biologically inactive carboxyl-terminal fragments. The carboxyl-terminal fragments are cleared only by glomerular filtration, while the bioactive intact PTH and amino-terminal fragments are also metabolically degraded in the liver and other tissues. The half-life of the carboxyl- terminal fragments increases dramatically in patients with renal failure. Thus, the measurement of intact PTH correlates best with the hormone production and biological activity.

2.2 Clinical application

The measurement of intact hPTH is used to establish the diagnosis of primary hyperparathyroidism by demonstrating elevated serum levels of bioactive PTH. It allows documenting the occurrence of secondary hyperparathyroidism in patients with Vit.D deficiency, intestinal malabsorption, or renal failure. In this last situation, the absence of interference with the inactive carboxyl-terminal fragments is especially valuable. The specificity and high sensitivity of the assay also allows differentiating clearly low serum PTH levels in hypoparathyroidism or in tumor-induced hypercalcaemia.

3. TEST PRINCIPLE

The BioVendor hPTH-ELISA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on breakable microtiterplates. Calibrators and samples react with the capture polyclonal antibodies (PAb, goat anti 1-34 PTH fragment) coated on microtiter well. After incubation, the excess of antigen is removed by washing. Then monoclonal antibodies (MAb, mouse anti 44-68 PTH fragment) labelled with horseradish peroxidase (HRP) are added. After an incubation period allowing the formation of a sandwich: coated PAbs – human PTH – MAb – HRP, the microtiterplate is washed to remove unbound enzyme labelled antibody. Bound enzymelabelled antibody is measured through a chromogenic reaction. The chromogenic solution (TMB) is added and incubated. The reaction is stopped with the addition of Stop Solution and the microtiterplate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is proportional to the PTH concentration.
A calibration curve is plotted and PTH concentration in samples is determined by interpolation from the calibration curve. The use of the ELISA reader (linearity up to 3 OD units) and a sophisticated data reduction method (polychromatic data reduction) result in a high sensitivity in the low range and in an extended calibration range.

4. REAGENT SUPPLIED

Reagents	96 tests Kit	Reconstitution
Microtiterplate with 96 anti PTH (polyclonal antibodies) coated breakable wells	96 wells	**Ready** for use
Conjugate: HRP labelled anti-PTH (monoclonal antibodies) in TRISmaleate buffer with bovine serum albumin, thymol and sheep serum	1 vial 13 ml	**Ready** for use
Zero calibrator in human plasma and thymol	1 vial lyophilized	**Add** 3 ml distilled water
Calibrator N = 1 to 5 (see exact values on vial labels) in human plasma and thymol	5 vials lyophilized	**Add** 1 ml distilled water
Wash Solution (NaCl-Tween20)	1 vial 25 ml	**Dilute** 28 x with distilled water (use a magnetic stirrer).
Controls - N = 1 or 2 in human plasma with thymol	2 vials lyophilized	**Add** 1 ml distilled water
Incubation Buffer with EDTA, Benzamidin and azide (< 0.1%)	1 vial 6 ml	**Ready** for use
Chromogenic Solution (TMB : Tetramethylbenzydine)	1 vial 13 ml	**Ready** for use
Stopping reagent H_2SO_4 0.2 M	2 vials 13 ml	**Ready** for use

Note:

1. Use the zero calibrator for sample dilutions.
2. 1 pg of the calibrator preparation is equivalent to 1 pg of a synthetic PTH (1-84) from the Japanese Peptide Institute.

5. MATERIAL REQUIRED BUT NOT SUPPLIED

The following material is required but not provided in the kit:

- High quality distilled water
- Pipettes for delivery of: 50 µl, 100 µl, 200 µl, 1 ml, 2 ml and 3 ml (the use of accurate pipettes with disposable plastic tips is recommended)
- Vortex mixer
- Magnetic stirrer
- Horizontal microtiterplate shaker capable of 700 rpm ± 100 rpm
- Washer for microtiterplates
- Microtiterplate reader capable of reading at 450 nm, 490 nm and 650 nm (in case of polychromatic reading) or capable of reading at 450 nm and 650 nm (monochromatic reading)
- Optional equipment: The ELISA-AID™ necessary to read the plate according to polychromatic reading (see paragraph XI.A.) can be purchased from Robert Maciels Associates, Inc. Mass. 0.2174 USA.

6. PREPARATION OF REAGENTS

6.1 Calibrators:

Reconstitute the zero calibrator with 3 ml distilled water and other calibrators with 1 ml distilled water.

6.2 Controls

Reconstitute the controls with 1 ml distilled water.

6.3 Working Wash solution

Prepare an adequate volume of Working Wash solution by adding 27 volumes of distilled water to 1 volume of Wash Solution (28x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

7. STORAGE AND EXSPIRATION

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the vial label, if kept at 2 to 8°C.
- Unused strips must be stored, at 2-8°C, in a sealed bag containing a desiccant until expiration date.
- After reconstitution, calibrators and controls should be frozen immediately after use and kept at –20°C for 3 months. Avoid successive freeze thaw cycles.
- The concentrated Wash Solution is stable at room temperature until expiration date.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, the conjugate is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

8. PREPARATION OF SAMPLES

- Blood samples should be promptly separated from the blood cells.
- Serum and plasma must be kept at 2 - 8°C.
- If the test is not run within 8 hours, storage in aliquots at -20°C is recommended. Avoid subsequent freeze thaw cycles.
- Prior to use, all samples should be at room temperature. It is recommended to vortex the samples before use.
- It is advisable to assay serum samples.
- Do not use haemolysed samples.

9. ASSAY PROCEDURE

9.1 Handling notes

Do not use the kit or components beyond expiry date. Do not mix materials from different kit lots.
Bring all the reagents to room temperature prior to use. Thoroughly mix all reagents and samples by gentle agitation or swirling. Perform calibrators, controls and samples in duplicate.
Vertical alignment is recommended.
Use a clean plastic container to prepare the Wash Solution. In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample. For the dispensing of the Chromogenic Solution and the Stop Solution avoid pipettes with metal parts.
High precision pipettes or automated pipetting equipment will improve the precision.
Respect the incubation times.
To avoid drift, the time between pipetting of the first calibrator and the last sample must be limited to the time mentioned in section XIII paragraph E (Time delay).
Prepare a calibration curve for each run, do not use data from previous runs.
Dispense the Chromogenic Solution within 15 minutes following the washing of the microtiterplate.
During incubation with Chromogenic Solution, avoid direct sunlight on the microtiterplate.

9.2 Procedure

1. Select the required number of strips for the run. The unused strips should be resealed in the bag with a desiccant and stored at 2-8°C.
2. Secure the strips into the holding frame.
3. Pipette 50 µl of Incubation Buffer into all wells.
4. Pipette 200 µl of each Calibrator, Control and Sample into the appropriate wells.
5. Incubate for 2 hours at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm.
6. Aspirate the liquid from each well.
7. Wash the plate 4 times by:
   - Dispensing 0.4 ml of Wash Solution into each well
   - Aspirating the content of each well
8. Pipette 100 µl of anti-PTH-HRP conjugate into all the wells.
9. Incubate for 1 hour at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm.
10. Aspirate the liquid from each well.
11. Wash the plate 4 times by:
    - Dispensing 0.4 ml of Wash Solution into each well
    - Aspirating the content of each well
12. Pipette 100 µl of the Chromogenic Solution into each well within 15 minutes following the washing step.
13. Incubate the microtiterplate for 30 minutes at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm, avoid direct sunlight.
14. Pipette 200 µl of Stop Solution into each well.

15. Read the absorbancies at 450 nm and 490 nm (reference filter 630 nm or 650 nm) within 1 hour and calculate the results as described in section XI.

10. CALCULATIONS

10.1 Polychromatic Reading:

1. In this case, the ELISA-AID™ software will do the data processing.
2. The plate is first read at 450 nm against a reference filter set at 650 nm (or 630 nm).
3. A second reading is performed at 490 nm against the same reference filter.
4. The ELISA-AID™ Software will drive the reader automatically and will integrate both readings into a polychromatic model. This technique can generate OD's up to 10.
5. The principle of polychromatic data processing is as follows:
   - Xi = OD at 450 nm
   - Yi = OD at 490 nm
   - Using a standard unweighted linear regression, the parameters A & B are calculated: Y = A*X – B
   - If Xi < 3 OD units, then X calculated = Xi
   - If Xi > 3 OD units, then X calculated = (Yi-B)/A
   - A 4 parameter logistic curve fitting is used to build up the calibration curve.
   - The PTH concentration in samples is determined by interpolation on the calibration curve.

10.2 Bichromatic Reading

1. Read the plate at 450 nm against a reference filter set at 650 nm (or 630 nm).
2. Calculate the mean of duplicate determinations.
3. Plot the OD values (ordinate) for each calibrator against the corresponding concentration of PTH (abscissa) and draw a calibration curve through the calibrator points.
4. Read the concentration for each control and sample by interpolation on the calibration curve.
5. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4 parameter logistic function curve fitting is recommended.

11. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

hPTH-ELISA		OD units Polychromatic model
Calibrator	0 pg/ml	0.027
	38 pg/ml	0.082
	94 pg/ml	0.165
	341 pg/ml	0.580
	993 pg/ml	1.699
	1955 pg/ml	3.139

12. PERFORMANCE CHARACTERISTICS AND LIMITATIONS

12.1 Detection Limit

Twenty zero calibrators were assayed along with a set of other calibrators. The detection limit, defined as the apparent concentration two standard deviations above the average OD at zero binding, was 0.8 pg/ml.

12.2 Specificity

Cross-reactive hormones or fragments were added to the zero calibrator and two serum samples with different hPTH concentrations. The apparent PTH response was measured.

Cross-reactant	No significant interference up to
PTH 1-34 synthetic fragment, human	1000 pg/ml
PTH 44-68 synthetic fragment, human	20000 pg/ml
PTH 53-84 synthetic fragment, human	20000 pg/ml
PTH 73-84 synthetic fragment, human	100000 pg/ml
PTH-related protein 1-34 synthetic fragment, human	100000 pg/ml

12.3 Precision

INTRA ASSAY				INTER ASSAY			
Serum	**N**	**<X> ± SD (pg/ml)**	**CV (%)**	**Serum**	**N**	**<X> ± SD (pg/ml)**	**CV (%)**
A	20	780.1 ± 26.5	3.4	A	10 10	244.6 ± 9.8	4.0
B	20	316.6 ± 6.8	2.1	B		1297 ± 56.4	4.3

SD: Standard Deviation; CV: Coefficient of variation

12.4 Accuracy

12.4.1 RECOVERY TEST

Sample	Added PTH (pg/ml)	Recovered PTH (pg/ml)	Recovery (%)
Serum	497	473	95
Heparin plasma	497	502	96
EDTA plasma	497	500	100

12.4.2 DILUTION TEST

Sample	Dilution	Theoretical Concent. (pg/ml)	Measured Concent. (pg/ml)
Serum	1/1	-	1815
	1/2	908	831
	1/4	454	425
	1/8	227	252

Samples were diluted with zero calibrator.

12.5 Time delay between last calibrator and sample dispensing

As shown hereafter, assay results (pg/ml) remain accurate even when a sample is dispensed 60 minutes after the calibrators have been added to the coated wells.

	TIME DELAY				
	T0	15 min	30 min	45 min	60 min
S1	158	146	166	144	137
S2	77	72	70	67	72
S3	328	320	323	342	357
S4	260	250	250	258	251

13. INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots. Controls which contain azide will interfere with the enzymatic reaction and cannot be used.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises.
- It is recommended that Controls be routinely assayed as unknown samples to measure assay variability. The performance of the assay should be monitored with quality control charts of the controls.
- It is good practise to check visually the curve fit selected by the computer.

14. REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.

	N	Median (pg/ml)	Range (pg/ml)
Normal patients	156	29	16 - 46
Hyperparathyroidism	64	291	106 - 1000
Hypoparathyroidism	11	0	0 – 6.4

The range is based on 5% to 95% percentiles.

15. PRECAUTIONS AND WARNINGS

15.1 Safety

For *in vitro* diagnostic use only.
The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HBsAg, anti- HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.
Avoid any skin contact with all reagents, Stop Solution contains H_2SO_4, the chromogen contains TMB. In case of contact, wash thoroughly with water. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves. For more information, refer to the MSDS.

16. REFERENCES

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Characteristics of the assay and analysis of circulating hormone forms".
J. Clin. Invest., 65:1309.

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6. BOUILLON R., COOPMANS W., DE GROOTE D.E.H., RADOUX D., ELIARD P.H. (1990) "Immunoradiometric assay of Parathyrin with polyclonal and monoclonal region specific antibodies".
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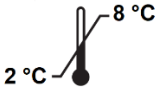
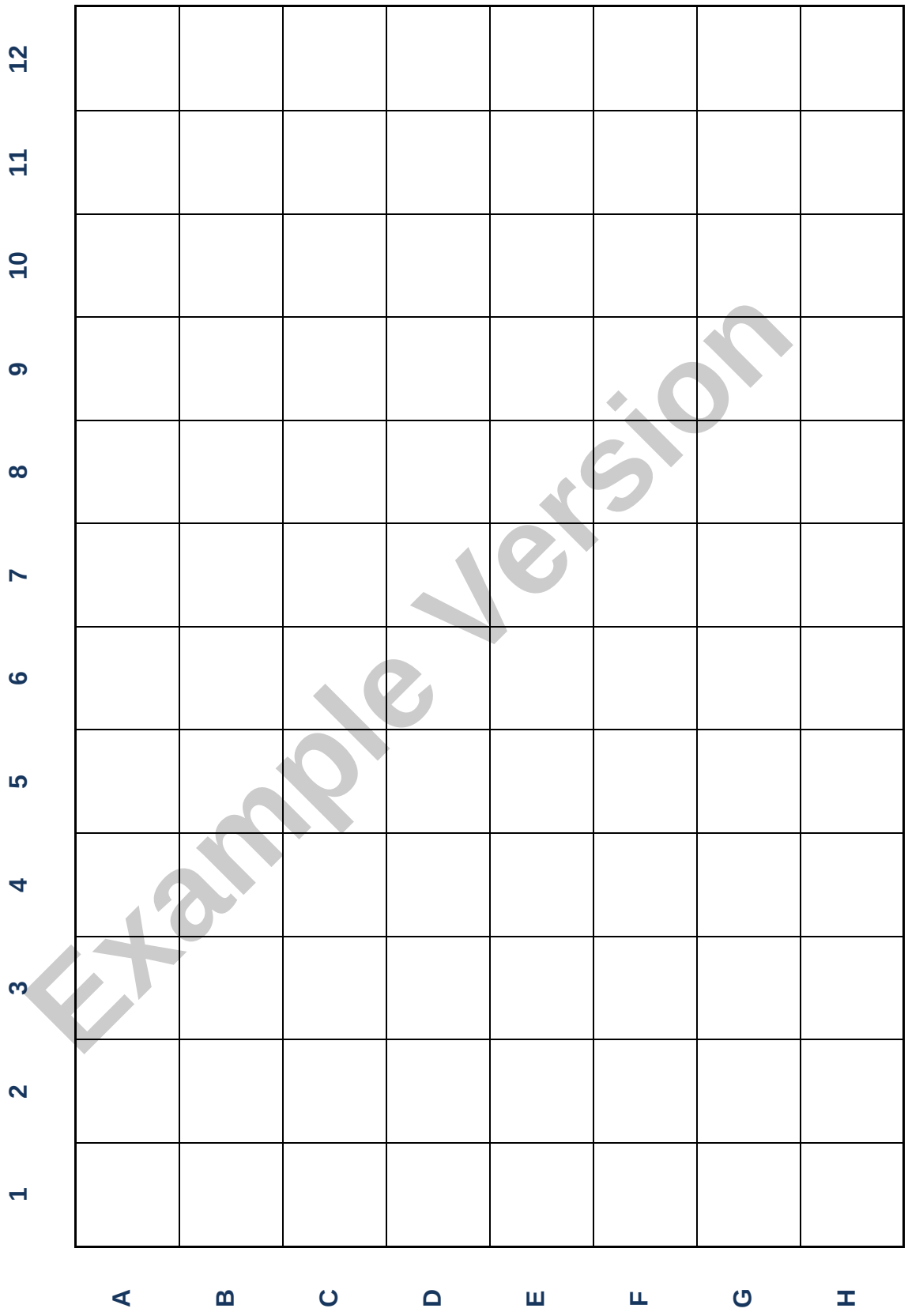
17. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks

18. ASSAY PROCEDURE - SUMMARY

	CALIBRATORS (µl)	SAMPLE(S) CONTROLS (µl)
Incubation buffer Calibrators (0-5) Samples, Controls	50 200 -	50 - 200
Incubate for 2 hours at room temperature with continuous shaking at 700 rpm. Aspirate the contents of each well. Wash 4 times with 400 µl of Wash Solution and aspirate.		
Anti-PTH-HRP	100	100
Incubate for 1 hour at room temperature with continuous shaking at 700 rpm. Aspirate the contents of each well. Wash 4 times with 400 µl of Wash Solution and aspirate.		
Chromogenic Solution	100	100
Incubate for 30 min at room temperature with continuous shaking at 700 rpm.		
Stop Solution	200	200
Read on a microtiterplate reader and record the absorbance of each well at 450 nm (versus 630 or 650 nm) and 490 nm (versus 630 or 650 nm)		



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