Rat NT-proBNP ELISA

Cat.No. BI-1204R
12x8 Tests

IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF RAT N-TERMINAL PRO-BRAIN NATRIURETIC PEPTIDE (NT-proBNP) IN SERUM OR PLASMA

For research use only. Not for use in diagnostic procedures.

This kit was developed and manufactured by:

BIOMEDICA

This package insert must be read entirely before using this product.

Detailed information on the rat NT-proBNP ELISA, e.g. assay validation data and stability data, is available on our website.

Related Products

- Human NT-proBNP ELISA (#SK-1204)
- NT-proANP ELISA (#BI-20892) - human & suitable for rat/mouse samples
- NT-proCNP ELISA (#BI-20812)

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INTRODUCTION

NT-proBNP PROTEIN

BNP has a key role in cardiovascular homeostasis with biological actions including natriuresis, diuresis, vasorelaxation, and inhibition of renin and aldosterone secretion. A high concentration of BNP in the bloodstream is indicative of heart failure.

BNP is mainly expressed by the ventricular myocardium in response to volume overload and increased filling pressure. It is synthesized and secreted by cardiomyocytes. Mature BNP consists of 108 amino acids (proBNP or BNP-108). proBNP is cleaved during secretion in a 1:1 ratio resulting in physiologically active BNP-32 and the biologically inactive 76 amino acid NT-proBNP (http://www.uniprot.org/uniprot/P16860#PRO_0000001532).

NT-proBNP (1-76) has greater plasma stability and a much longer biological half-life (90-120 minutes) than BNP, being considered as the preferred laboratory marker.

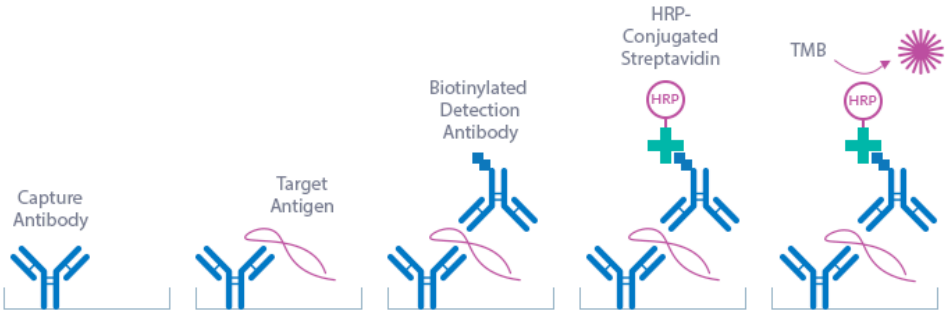
AREAS OF INTEREST

- Heart failure - cardiac impairment, acute myocardial infarction
- Left ventricular dysfunction
- Autoimmune disease - rheumatoid arthritis, psoriasis
- Renal failure, chronic kidney disease
- Obesity and diabetes

ASSAY PRINCIPLE

The Biomedica rat NT-proBNP ELISA kit is a sandwich enzyme immunoassay that has been optimized and fully validated for the quantitative determination of rat NT-proBNP in serum or plasma samples. The rat NT-proBNP ELISA assay recognizes both natural and recombinant rat NT-proBNP.

The figure below explains the principle of the rat NT-proBNP sandwich ELISA:



ASSAY PRINCIPLE CONTINUED

In a first step, ASYBUF (Assay buffer), STD/CTRL/Sample (Standard, Control, Sample) and detection antibody (biotinylated polyclonal anti-rat NT-proBNP antibody, AB) are pipetted into the respective wells of the microtiter strips, which are pre-coated with a polyclonal anti-rat NT-proBNP antibody. Rat NT-proBNP present in the sample binds to the pre-coated antibody in the well and forms a sandwich with the detection antibody.

After a first wash step, which removes non-specifically unbound material, the conjugate (Streptavidin-HRPO) is added and reacts with the detection antibody. After another washing step, the substrate (TMB, tetramethylbenzidine) is pipetted into the wells.

The enzyme catalysed color change of the substrate is directly proportional to the amount of rat NT-proBNP present in the sample. This color change is detectable with a standard microtiter plate ELISA reader. The concentration of rat NT-proBNP in the sample is determined directly from the dose response curve. The kit utilizes recombinant rat NT-proBNP as a calibrator.

ELISA KIT COMPONENTS

All reagents supplied in the rat NT-proBNP ELISA kit are stable at 2-8°C until the expiry date stated on the label of each reagent.

CONTENT	DESCRIPTION	QUANTITY
PLATE	Detachable microtiter strips pre-coated with polyclonal anti-rat NT-proBNP antibody, packed in an aluminum bag with desiccant	12 x 8 tests
WASHBUF	Wash buffer concentrate 20x, natural cap	1 x 50 ml
ASYBUF	Assay buffer, **red** cap, ready to use	1 x 7 ml
STOCK STD	Stock standard containing recombinant rat NT-proBNP protein, concentration 3200 pg/ml, **red** cap, lyophilised	1 vial
CTRL	Control, **yellow** cap, lyophilized, exact concentration is stated on label	1 vial
AB	Polyclonal anti-rat NT-proBNP antibody, biotinylated, **green** cap, ready to use	1 x 7 ml
CONJ	Conjugate (streptavidin-HRPO), **brown** cap, ready to use	1 x 13 ml
SUB	Substrate (TMB solution), **blue** cap, ready to use	1 x 13 ml
STOP	STOP solution, white cap, ready to use	1 x 7 ml

ADDITIONAL KIT COMPONENTS

Three self-adhesive plastic films
Quality control protocol
Instruction for use
Plate layout sheet

OTHER SUPPLIES REQUIRED

Precision and multichannel pipettes calibrated to deliver 10 µl, 50 µl, 100 µl, and disposable tips.
Distilled or deionized water.
Plate washer is recommended for washing, alternative a multichannel pipette or manifold dispenser.
Refrigerator with 4°C (2-8°C).
A microplate reader capable of measuring absorbance at 450nm (optionally with a correction wavelength at 630nm).
Software for the calculation of results or, alternatively, graph paper.
Polypropylene tubes for standard curve preparation

SAMPLE COLLECTION AND STORAGE

Serum and plasma samples are suitable for use in this assay. Do not change sample type during studies. The sample collection and storage conditions listed are intended as general guidelines.

SERUM & PLASMA

Collect venous blood samples by using standardized blood collection tubes. Perform plasma or serum separation by centrifugation according to supplier's instructions of the blood collection devices. Assay the acquired samples immediately or aliquot and store at -25°C or lower. Samples are stable for up to five freeze-thaw cycles.

Lipemic or haemolysed samples may give erroneous results. Samples should be mixed well before assaying. We recommend duplicates for all values.

Samples with values above STD7 (3200 pg/ml) can be diluted with ASYBUF (Assay buffer).

REAGENT PREPARATION

WASH BUFFER

1.	Bring the WASHBUF concentrate to room temperature. Crystals in the buffer concentrate will dissolve at room temperature (18-26°C).
2.	Dilute the WASHBUF concentrate 1:20, e.g. 50 ml WASHBUF + 950 ml distilled or deionized water. Only use diluted WASHBUF when performing the assay.

The diluted WASHBUF is stable up to one month at 4°C (2-8°C).

REAGENT PREPARATION CONTINUED

STOCK STD (STOCK STANDARD) & CTRL (CONTROL)

Bring all reagents to room temperature before use.

1.	Pipette **200 µl** of distilled or deionized water into stock standard (STOCK STD) and control (CTRL) vial. The exact concentration is printed on the label of each vial.
2.	Leave at room temperature (18-26°C) for 15 min. Vortex gently.

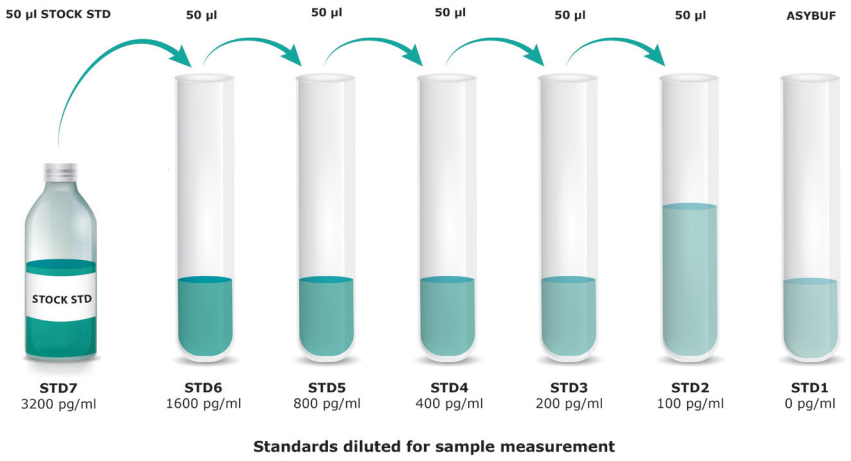
Reconstituted STOCK STD and CTRL are stable at -25°C or lower until the expiry date stated on the label. STOCK STD and CTRL are stable for up to five freeze-thaw cycles.

The standards and control provided in the kit are suitable for serum and plasma samples.

Preparation of the standard curve: Always mix each tube thoroughly before the next step!

1.	Use polypropylene tubes and mark them as STD6 to STD2.
2.	Mark STOCK STD as STD7.
3.	Pipette 50 µl of ASYBUF into each tube marked as STD6 to STD2.
4.	Prepare a two-fold serial dilution to obtain STD6 to STD2. Pipette 50 µl of the reconstituted STOCK STD (=STD7) into the tube labelled STD6. Mix thoroughly. Continue serial dilutions for STD5, STD4, STD3 and STD2.
5.	ASYBUF serves as the zero standard (=STD1, 0 pg/ml).

Graph: Preparation of standards 7 to 1 (STD7-STD1)



ASSAY PROTOCOL

Read the entire instructions for use before beginning the assay.

All reagents and samples must be at room temperature (18-26°C) before use in the assay.

Mix samples gently to ensure the samples are homogenous. We recommend performing duplicate measurements for all samples, standards and control.

Mark position for STD/CTRL/SAMPLE (standard/control/sample) on the plate layout sheet.

Take microtiter strips out of the aluminum bag. Store unused strips with desiccant at 4°C (2-8°C) in the aluminum bag. Strips are stable until the expiry date stated on the label.

1.	Pipette **50 µl** ASYBUF (Assay buffer, **red** cap) into each well.
2.	Add **10 µl** of STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective well. Swirl gently.
3.	Add **50 µl** AB (biotinylated anti-rat NT-proBNP antibody, **green** cap) into each well. Swirl gently.
4.	Cover tightly and incubate for **2 hours** at room temperature (18-26°C).
5.	Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer, transparent cap). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
6.	Add **100 µl** CONJ (Conjugate, **brown** cap) into each well. Swirl gently.
7.	Cover tightly and incubate for **1 hour** at room temperature (18-26°C).
8.	Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer, transparent cap). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
9.	Add **100 µl** SUB (Substrate, **blue** cap) into each well. Swirl gently.
10.	Cover tightly and incubate for **30 min** at room temperature (18-26°C) in the dark.
11.	Add **50 µl** STOP (Stop solution, white cap) into each well. Swirl gently.
12.	Measure absorbance immediately at 450 nm with reference 630 nm, if available.

PRECAUTIONS

Liquid reagents in this assay contain ≤0.1% Proclin 950 as a preservative. Proclin 950 is not toxic in concentrations used in this kit but may cause allergic skin reactions – avoid contact with skin or eyes.
Do not pipette by mouth.
Do not eat, drink, smoke or apply cosmetics where reagents are used.
Refer to the Material Safety Data Sheet (MSDS) available for download at www.bmgrp.com.
Avoid all contact with reagents by using protective gloves, clothing and eye protection.
Sulfuric acid contained in the STOP solution may cause irritations to eyes and skin. Avoid contact with skin and mucous membrane. Flush with water if contact occurs!

TECHNICAL HINTS

Do not mix or substitute reagents with those from other lots or sources.
Do not mix stoppers and caps from different reagents or use reagents between lots.
Do not use reagents beyond the expiration date.
Protect reagents from direct sunlight.
Substrate solution should remain colorless until added to the plate.
Seal plates properly with the self-adhesive films during incubation steps to ensure accurate results.
Avoid foaming when mixing reagents.

CALCULATION OF RESULTS

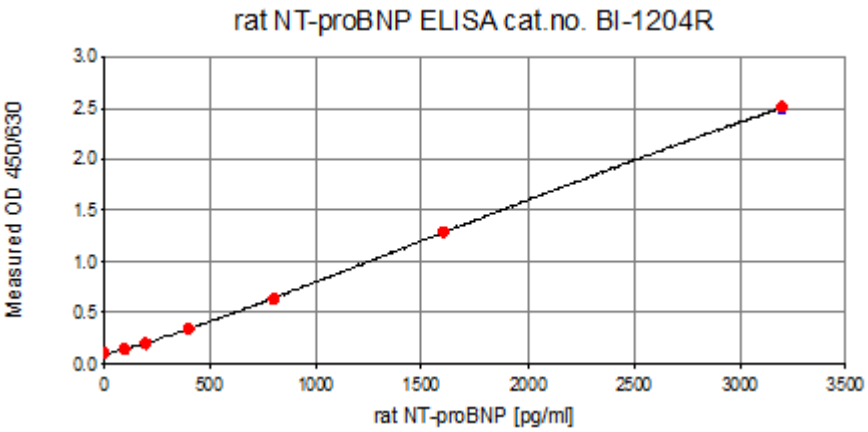
Construct a standard curve from the absorbance read-outs of the standards using commercially available software capable of generating a four-parameter logistic (4-PL) fit. Alternatively, plot the standards' concentration on the x-axis against the mean absorbance for each standard on the y-axis and draw a best fit curve through the points on the graph. Curve fitting algorithms other than 4-PL have not been validated and will need to be evaluated by the user.

Samples with analyte concentrations outside of the calibration range of the assay (3200 pg/ml) should be diluted with assay buffer.

Concentrations of high-measuring samples that have been diluted during sample preparation must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve and the displayed OD values are for demonstration only. A standard curve should be generated for each assay run.



STANDARD	Rat NT-proBNP pg/ml	OD			CV%
		#1	#2	AVERAGE	
STD1	0	0.095	0.105	0.100	7
STD2	100	0.148	0.156	0.152	4
STD3	200	0.192	0.204	0.198	4
STD4	400	0.353	0.341	0.347	2
STD5	800	0.641	0.638	0.640	0
STD6	1600	1.390	1.238	1.337	6
STD7	3200	2.520	2.488	2.504	1

The quality control protocol supplied with the kit shows the results of the final release QC for each kit at the production date. ODs obtained by customers may differ due to various influences including a normal decrease of signal intensity throughout shelf life. However, this does not affect the validity of the results provided an OD of 1.0 or higher is obtained for the standard with the highest concentration and the measured control value falls into its target range (see label).

ASSAY CHARACTERISTICS OVERVIEW

Method	Sandwich ELISA, HRPO/TMB, 12x8-well detachable strips				
Sample type(s)	Rat serum, plasma				
Sample volume	10 µl sample / well				
Standard range	0 – 3200 pg/ml (0 / 100 / 200 / 400 / 800 / 1600 / 3200 pg/ml)				
Sensitivity	LOD: 21 pg/ml; LLOQ: 50 pg/ml				
Assay time	2 h / 1 h / 30 min				
Precision			**n**	**Average % CV**	
	Within-run		3	≤6	
	In-between-run		9	≤4	
Accuracy (Spike/Recovery of rec rat NT-proBNP)			**n**	**Average % recovery**	
				+1600 pg/ml	**+400 pg/ml**
	Rat serum		6	90	86
	Rat plasma		3	88	81
Parallelism of endogenous rat NT-proBNP		**n**	**Average % of expected dilution**		
			1+1	**1+3**	**1+7**
	Rat serum	6	106	116	113
Specificity	This assay recognizes recombinant and endogenous (natural) rat NT-proBNP.				
Use	Research use only.				
Median rat NT-proBNP values in various cohorts			**n**	**Median rat NT-proBNP (pg/ml)**	
	Rat serum *		6	74	
	Rat plasma *		14	22	
	Rat serum (CTRL group - disease model AN)		5	379	
	Rat serum (disease model AN)		8	706	
	Rat serum (CTRL group - disease model RMR)		4	110	
	Rat serum (disease model RMR)		7	708	

**values derive from rat strains with different genetic backgrounds*
Abbreviations: CTRL: control; AD: Adriamycin nephropathy; RMR: renal mass reduction

PRECISION

WITHIN-RUN PRECISION

Within-run / intra-assay precision was tested by measuring two samples of known concentrations three times within one rat NT-proBNP ELISA kit lot by one operator.

IN-BETWEEN-RUN PRECISION

In-between-run / inter-assay precision was tested by measuring two samples of known concentrations nine times within different rat NT-proBNP ELISA lots by two different operators.

Within-run (n=3)	Sample 1	Sample 2	In-between-run (n=9)	Sample 1	Sample 2
Mean (pg/ml)	153	1600	Mean (pg/ml)	199	1603
SD (pg/ml)	9.8	27.0	SD (pg/ml)	8.8	344.4
CV (%)	6	2	CV (%)	4	2

SENSITIVITY

LOWER LIMIT OF DETECTION (LOD) & LOWER LIMIT OF QUANTIFICATION (LLOQ)

The LOD is defined as the mean back-calculated concentration of standard 1 (0 pg/ml of rat NT-proBNP, five independent measurements) plus three times the standard deviation of the measurements.

The LLOQ is defined as the lowest concentration at which an analyte can be accurately quantified. To determine the LLOQ, standard 2, i.e., the lowest standard containing rat NT-proBNP is diluted, measured five times and its concentration back calculated.

The following values were determined for the rat NT-proBNP ELISA:

LOD	21 pg/ml
LLOQ	50 pg/ml

CALIBRATION

The rat NT-proBNP immunoassay is calibrated against recombinant rat NT-proBNP peptide.

SAMPLE VALUES

SERUM/PLASMA

Rat serum/plasma samples derived from rat strains with different genetic backgrounds.
Rat NT-proBNP reference values may differ from other rat strains.

		Rat NT-proBNP (pg/ml)				
Sample Matrix	**n**	**Mean**	**Median**	**Minimum**	**Maximum**	**% Detectable**
Serum	6	82	74	0	169	83
Plasma*	14	47	22	0	185	86

**EDTA and citrate plasma*

It is recommended to establish the normal range for each laboratory.

SPECIFICITY

This rat NT-proBNP ELISA recognizes recombinant and endogenous (natural) rat NT-proBNP.

LITERATURE

1. Heart Failure Association of the European Society of Cardiology practical guidance on the use of natriuretic peptide concentrations. Mueller C et al., Eur J Heart Fail, 21(6):715-731 (2019).
2. Utility of the Amino-Terminal Fragment of Pro Brain Natriuretic Peptide in Plasma. For the Evaluation of Cardiac Dysfunction in elderly Patients in Primary Health Care. Alehagen U et al., Clin Chem, 49:8; 1337-1346 (2003).
3. Head-to-head comparison of N-terminal pro-brain natriuretic peptide, brain natriuretic peptide and N-terminal proatrial natriuretic peptide in diagnosing left ventricular dysfunction. Hammerer-Lercher et al., Clin Chim Acta, 310(2),193-197 (2001).
4. Molecular Signaling Mechanisms and Function of Natriuretic Peptide Receptor-A in the Pathophysiology of Cardiovascular Homeostasis. Pandey KN et al., Front Physiol, 19;12:693099 (2021).
5. NT-ProBNP and mortality across the spectrum of glucose tolerance in the general US population. Ciardullo S et al., Cardiovasc Diabetol, 7;21(1):236 (2022).
6. Plasma pro-B-type natriuretic peptide in the general population: screening for left ventricular hypertrophy and systolic dysfunction. Goetze JP et al., Eur Heart J, 27: 3004-3010 (2006).
7. N-terminal pro brain natriuretic peptide reference values in community-dwelling older adults. Braisch U et al., ESC Heart Fail, 9(3):1703-1712 (2022).

SYMBOLS

	Expiry date / Verfallsdatum / Date de péremption / Data di scadenza /Fecha de caducidad / Data de validade / Uiterste gebruiksdatum / Udløbsdato / Utgångsdatum / Termin Ważności / Lejárati idö / Doba exspirácie / Doba exspirace
	Consider instructions for use / Bitte Gebrauchsanweisung beachten / Consultez la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las instrucciones de utilización / Consulte as instruções de utilização / Raadpleeg de gebruiksaanwijzing / Se brugsanvisningen / Läs anvisningarna före användning / Proszę przeczytać instrukcję wykonania / Vegyük figyelembe a használati utasításban foglaltakat / Postupujte podl'a pokynov na použitie / Postupujte dle návodu k použití
	Lot-Batch Number / Charge-Chargennummer / Lot-Code du lot / Lotto-Numero di lotto / Lote-Código de lote / Lote-Código do lote / Lot-Partijnummer / Lot-Batchkode / Lot-Satskod / Numer serii / Lot-Batch szám / Číslo šarže / Číslo šarže
	Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por / Vervaardigd door / Fabrikation af / Tillverkad av / Wyprodukowane pr / Gyártotta / Vyrobené / Vyrobeno
	Catalogue Number / Bestellnummer / Numéro de référence / Numero di riferimento / Número de referencia / Número de referência / Referentienummer / Referencenummer / Katalognummer / Numer katalogowy / Katalógusszám / Katalógové číslo / Katalogové číslo
	Store at between / Lagerung bei zwischen / Conserver à entre / Conservare a tra / Conservar a temp. entre / Armazene a entre / Bewaar bij tussen / Opbevares mellem / Förvaras vid / Przechowywać w / Tároljuk ..... között / Skladujte v rozsahu / Skladujte v rozmezí
	Contains sufficient for x tests / Inhalt ausreichend für x Tests / Contient suffisant pour x tests / Contenuto sufficiente per x test / Contiene suficiente para x pruebas / Contém suficiente para x testes / Bevat voldoende voor x bepalingen / Indeholder tilstrækkeligt til x prøver / Innehållet räcker till x analyser / Zawartość na x testów / Tartalma X teszt elvégzésére elegendö / Obsahuje materiál pre x testov / Obsahuje materiál pro x testu

ASSAY PROTOCOL & CHECKLIST - FOR ALL SAMPLE TYPES

Rat NT-proBNP ELISA - # BI-1204R

REAGENT PREPARATION

	Read the entire instruction for use before beginning the assay.
☐	Bring all reagents to room temperature (18-26°C).
☐	Prepare reagents and samples as instructed.
☐	Bring unused and prepared components to the storage temperature mentioned in the package insert.
☐	Take microtiter strips out of the aluminum bag and mark STD, CTRL, and SAMPLE positions on the plate layout sheet.

ASSAY PROCEDURE

☐	1. Pipette **50 µl** ASYBUF (Assay buffer, **red** cap) into each well.
☐	2. Add **10 µl of STD/SAMPLE/CTRL** (Standard/Sample/Control) in duplicate into respective well. Swirl gently.
☐	3. Add **50 µl** AB (biotinylated anti-rat NT-proBNP antibody, **green** cap) into each well. Swirl gently.
☐	4. Cover tightly and **incubate for 2 hours** at room temperature (18-26°C).
☐	5. Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer, transparent cap). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
☐	6. Add **100 µl** CONJ (Conjugate, **brown** cap) into each well. Swirl gently.
☐	7. Cover tightly and **incubate for 1 hour** at room temperature (18-26°C).
☐	8. Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer, transparent cap). After final wash, remove remaining WASHBUF by strongly tapping plate against paper towel.
☐	9. Add **100 µl** SUB (Substrate, **blue** cap) into each well. Swirl gently.
☐	10. I**ncubate for 30 min** at room temperature (18-26°C) **in the dark.**
☐	11. Add **50 µl** STOP (Stop solution, white cap) into each well. Swirl gently.
☐	12. Measure absorbance immediately at 450 nm with reference 630 nm, if available.