Catalog Number: FIT-0100 96-well Test Kit

Functional Immunoassay Technology (FIT™) Glomerular Filtration Rate (GFR)

FIT-GFR™ Kit (Gd-DTPA)

Patents Pending

An enzyme immunoassay test kit for the determination of gadolinium-DTPA (Gd-DTPA) in serum and urine

• FOR RESEARCH USE ONLY •
• NOT FOR USE IN DIAGNOSTIC PROCEDURES •
• THIS PACKAGE INSERT MUST BE READ IN ITS ENTIRETY BEFORE USING THIS PRODUCT •

BioPhysics Assay Laboratory (BioPAL), Inc.
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PROPRIETARY NAME

Functional Immunoassay Technology (FIT™)
FIT-GFR™ Kit
BioPhysics Assay Laboratory (BioPAL), Inc.
Catalog Number: FIT-0100, 96-Well Test Kit

INTENDED USE

The FIT-GFR assay is an enzyme immunoassay used for the determination of Gd-DTPA in serum and urine. Following intravenous (IV) administration of Gd-DTPA, sequential blood and/or urine samples are collected from the subject. The rate of change in the concentration of Gd-DTPA measured in collected samples over time is used to calculate the GFR.

THE FIT-GFR™ ASSAY TEST KIT IS FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

REAGENTS AND MATERIALS SUPPLIED

Test kit reagents are provided as a set sufficient to perform 96 determinations. Some reagents require preparation on the day of assay. Kit should be stored at 4°C. Each kit includes:

1. **Goat anti-rabbit 96-well coated plate (one plate)**
   Catalog # FIT-0006
2. **Gadolinium-DTPA Concentrate (125 µl)**
   1 mg/ml Concentrate and should be diluted using Standard and Sample Diluent to appropriate concentrations to run a complete Standard Curve.
   Catalog # FIT-0103
3. **HRP-Gadolinium-DTPA (6 ml)**
   Store away from light.
   Catalog # FIT-0102
4. **Rabbit anti-Gadolinium-DTPA (6 ml)**
   Catalog # FIT-0101
5. **HRP Substrate Reagent (12 ml)**
   Proprietary solution provided ready to use. Store away from light.
   Catalog # FIT-0002
6. **HRP Stop Reagent (12 ml)**
   Proprietary solution provided ready to use.
   Catalog # FIT-0003
7. **Plate sealer (1 unit)**
   Catalog # FIT-0004
8. **FIT-GFR (Gd-DTPA) Kit Manual (1)**
   Catalog # FIT-0105
9. **Manual for Calculation of GFR in Research Subjects (1)**
   Catalog # FIT-0007
10. **Material Safety Data Sheets (1)**
    Catalog # FIT-0008

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ADDITIONAL KIT COMPONENTS AVAILABLE FOR PURCHASE

The following components are not included in the kit, but are necessary to run the assay. Researchers have the option of purchasing these reagents through BioPAL or preparing the reagents themselves. The reagent components are listed below.

1. **Standard and Sample Diluent (100ml)**
   Ready-to-use. Standard and Sample Diluent is composed of 0.1% bovine serum albumin, 0.01% thimerosal in PBS Buffer (0.0098M dibasic sodium phosphate, 0.138M sodium chloride, 0.00268M potassium chloride).
   Catalog # FIT-0001
2. **Wash Buffer Concentrate (100 ml)**
   Dilute $^{1}/_{10}$ with distilled water before using by adding 900 ml distilled water to the 100 ml Wash Buffer Concentration. Wash Buffer (unconcentrated) is composed of 0.05% Tween 20 in PBS Buffer (0.0098 M dibasic sodium phosphate, 0.138 M sodium chloride, 0.00268 M potassium chloride).
   Catalog # FIT-0005

MATERIALS REQUIRED TO RUN THE KIT, BUT NOT SUPPLIED

1. Precision pipettes with disposable tips to deliver 5 to 1000 µl volumes
2. 50-300 µl adjustable multi-channel pipette
3. Beaker, flask, cylinders necessary for preparation of reagents
4. 96-well plate washer/aspirator device
5. Mini-vortexer
6. Graph paper or computer software for data reduction
8. 96-well plate reader for measurement of absorbance at 450 nm
9. Labcor Non-Sterile Basins, 55 ml, Cat. No. 730-01 or equivalent
10. De-ionized or distilled water
11. Horizontal orbital microshaker

PRECAUTIONS

1. SUBJECT SPECIMENS AND ALL MATERIALS COMING INTO CONTACT WITH THEM SHOULD BE HANDLED AS IF CAPABLE OF TRANSMITTING INFECTION AND DISPOSED OF USING PROPER PRECAUTIONS. Wear disposable gloves while handling specimens and wash hands afterwards.
2. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
3. Do not pipette by mouth.
4. Reagents containing thimerosal may be toxic if ingested.
5. Avoid contact of Specimens, HRP-Gd-DTPA, Standard and Sample Diluent, HRP Substrate Reagent and HRP Stop Reagent with skin and mucous membranes. In case of contact, thoroughly wash the contaminated area.
6. Consult local regulations concerning disposal of kit reagents, assay materials and subject specimens.
7. Do not mix reagents from different kit lots.
8. Do not use kit components beyond expiration date.
9. Do not expose HRP Substrate Reagent to strong light during storage or incubation. Avoid contact of the HRP Substrate Reagent with oxidizing reagents. Do not allow HRP Substrate or Stop Reagents to contact any metal parts.

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10. When running multiple plates, a standard curve should be run with each individual plate.
11. Do not pour unused HRP Substrate Reagent back into original container. Take care not to contaminate the HRP Substrate Reagent. If the solution is blue before use, DO NOT USE.
12. Take precautions to avoid microbial contamination when opening and removing aliquots from primary vials.

STORAGE INSTRUCTIONS

1. Upon receipt, all kit reagents and components should be stored at 2-8°C.
2. DO NOT allow kit reagents to remain at room temperature for more than 1 hour before use.
3. All reagents must be brought to room temperature (24 ± 2°C) before starting the assay. Unused material must be returned to appropriate storage conditions.
4. Protect HRP-Gd-DTPA from exposure to light.

SPECIMEN COLLECTION AND HANDLING

There are a number of protocols a researcher can use to measure glomerular filtration rate (GFR). These protocols include, but are not limited to, the UV/P method, the complete blood clearance method, and single blood sample method. BioPAL's FIT-GFR (Gd-DTPA) kit will support all standard protocols for measuring glomerular filtration rate. However, the choice of method is the researcher's prerogative.

A venous blood sample is collected aseptically. Serum is suitable for use in the assay. **EDTA plasma cannot be used in this assay.** Remove the serum from the clot as soon as possible after clotting and separation. Whichever experimental protocol is chosen, it is recommended that the method be used consistently, as subtle changes in recovery may be seen.

Urine samples can be collected as a clean catch or collected by catheterization. Samples may be stored up to seven days at 2-8°C. Do not freeze samples.

PROCEDURAL NOTES

1. Assay each Gd-DTPA sample and standard in duplicate each time the assay is performed.
2. Since conditions can vary from assay to assay, a full standard curve must be established for every run and every plate.
3. Disposable pipette tips must be used to prevent cross contamination between reagents or specimens.
4. Reusable glassware must be washed and thoroughly rinsed free of all detergent before use.
5. Thorough washing and aspiration of wells following incubation is required.
6. Incubation times or temperatures other than those specified could cause erroneous results. Perform assay continuously, according to procedure and without interruption.

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INTERFERING CONDITIONS

1. SODIUM AZIDE INACTIVATES HORSERADISH PEROXIDASE.
   SPECIMENS CONTAINING SODIUM AZIDE SHOULD NOT BE USED IN THIS ASSAY.
2. Turbid specimens or those containing a visible precipitate must be centrifuged prior to use in this assay. DO NOT USE SERUM SPECIMENS WITH SUSPECTED MICROBIAL CONTAMINATION.

PROCEDURE

Reagent Preparation

Wash Buffer (1L)

Dilute Wash Buffer Concentrate (Cat. No.: FIT-0005) $^{1}/_{10}$ by adding 100 ml Wash Buffer Concentrate to 900 ml distilled water. Mix thoroughly.

Gd-DTPA Standards

Prepare 1 ml of the following dilutions of Gd-DTPA Concentrate (Cat. No.: FIT-0103, 1 mg/ml, using Standard and Sample Diluent:

0.3 µg/ml Gd-DTPA
0.1 µg/ml Gd-DTPA
0.03 µg/ml Gd-DTPA
0.01 µg/ml Gd-DTPA
0.003 µg/ml Gd-DTPA

ASSAY PROTOCOL

BRING ALL REAGENTS AND SAMPLES TO ROOM TEMPERATURE BEFORE USE.

Note: All standards and samples are run in duplicate. Design plate layout to accommodate the desired number of subject samples. For example, consider a standard UV/P protocol wherein 2 UV/P measurements will be obtained per subject. As a result, each subject will generate 4 serum and 2 urine samples (12 wells). An additional 16 wells will be needed for running blanks and standards (see Scheme 1, Page 8). Using this protocol and plate design, six subjects may be run on one plate.

1. Mix all reagents thoroughly without foaming before use.
2. As prepared above, pipette 50 µl Gd-DTPA Standards into each well in first two columns as designated by Scheme 1 (Page 8). DO NOT PIPETTE ANYTHING INTO THE WELLS DESIGNATED FOR BLANKS.
3. Pipette 50 µl of subject sample (serum or urine) per well, as designated by Scheme 1 (Page 8).
4. Pipette 50 µl HRP-Gd-DTPA (Cat. No.: FIT-0102) into all wells **except blanks.** Store conjugate in dark until needed.
5. Pipette 50 µl Rabbit anti-Gd-DTPA (Cat. No.: FIT-0101) into all wells **including blanks.**
6. Cover the plate with a Plate Sealer (Cat. No.: FIT-0004) and incubate on orbital shaker, operating at 500 rpm, for 1.5 hours.

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7. Upon completion of the 1.5-hour incubation period, remove and discard the plate sealer. Aspirate solution from all wells. Wash/aspirate with 350 µl Wash Buffer per well for a total of three times. Whack plate upside-down on a clean paper towel to remove residual liquid in wells.

8. Pipette 100 µl HRP Substrate Reagent (Cat. No.: FIT-0002) into all wells **including blanks.** Incubate substrate without shaking for 30 minutes.

9. Pipette 100 µl HRP Stop Reagent (Cat. No.: FIT-0003) into all wells **including blanks.** Tap plate gently to mix contents of each well.

10. Read absorbance of wells at 450 nm. The absorbance should be read as soon as possible after the completion of the assay, but may be read up to 30 minutes after addition of HRP Stop Reagent when wells are kept protected from light. Subtract the averaged blank from the averaged standards and unknowns.

    NOTE: It is important that HRP Stop Reagent be added to wells prior to reading at 450 nm. Addition of HRP Stop Reagent causes an increase in absorbance of the TMB component of the HRP Substrate Reagent and a shift in absorption spectrum.

11. Generate the standard curve by plotting the average absorbance obtained for each Standard concentration on the vertical (y) axis (normal scale) vs. the corresponding Gd-DTPA concentration (µg/ml) on the horizontal (x) axis (log scale).

    For best results, plot data using 4-parameter curve fitting statistical software. Manual plots on graphing paper can also be utilized, but are not recommended. Determination of the Gd-DTPA amount in each sample can be done by either (1) automatically generating value through curve-fitting software, (2) by using equation generated by software and program, such as Excel®, to fit unknown values into equation, or (3) by manually interpolating from the absorbance value (y-axis) to Gd-DTPA concentration (x-axis) using the standard curve.

    If the test sample was diluted multiply the interpolated value obtained from the standard curve by the dilution factor to calculate µg/ml of Gd-DTPA in the sample.

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Scheme 1: Example of a standard UV/P protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	blank	blank	serum t_1	serum t_1	serum t_3	serum t_3	urine t_1	urine t_1	serum t_1	serum t_1	serum t_3	serum t_3
B	**0.003**	**0.003**	serum t_2	serum t_2	serum t_4	serum t_4	urine t_2	urine t_2	serum t_2	serum t_2	serum t_4	serum t_4
C	**0.01**	**0.01**	serum t_3	serum t_3	urine t_1	urine t_1	serum t_1	serum t_1	serum t_3	serum t_3	urine t_1	urine t_1
D	**0.03**	**0.03**	serum t_4	serum t_4	urine t_2	urine t_2	serum t_2	serum t_2	serum t_4	serum t_4	urine t_2	urine t_2
E	**0.1**	**0.1**	urine t_1	urine t_1	serum t_1	serum t_1	serum t_3	serum t_3	urine t_1	urine t_1		
F	**0.3**	**0.3**	urine t_2	urine t_2	serum t_2	serum t_2	serum t_4	serum t_4	urine t_2	urine t_2		
G	Low Control	Low Control	serum t_1	serum t_1	serum t_3	serum t_3	urine t_1	urine t_1	serum t_1	serum t_1		
H	High Control	High Control	serum t_2	serum t_2	serum t_4	serum t_4	urine t_2	urine t_2	serum t_2	serum t_2		

Key:

Columns 1&2: Blanks and Standard Curve
Columns 3-12: Subject Samples
Grey Wells: Unused Wells

t_x: timepoint (4 serum timepoints, 2 urine timepoints; per Subject)

Subject 1 | Subject 2 | Subject 3 | Subject 4 | Subject 5 | Subject 6

RESULTS

Gd-DTPA values are expressed in micrograms per milliliter (µg/ml).

A. STANDARD CURVE

1. Record the absorbance at 450 nm for each standard well.
2. Calculate and record the mean absorbance for each pair of standard duplicates.
   a. Correct all values by subtracting the mean value for the blank from each standard mean absorbance value.
   b. Construct a standard curve by plotting the correct mean absorbance of each standard on the vertical (y) axis (normal) versus the corresponding gadolinium-DTPA concentration on the horizontal (x) axis (log). Use rectilinear graph paper or plotting software.
   c. Draw a point-to-point or smooth curve through the points on the graph or use a suitable curve-fitting program with 4-parameter fit to give best fit to the data.

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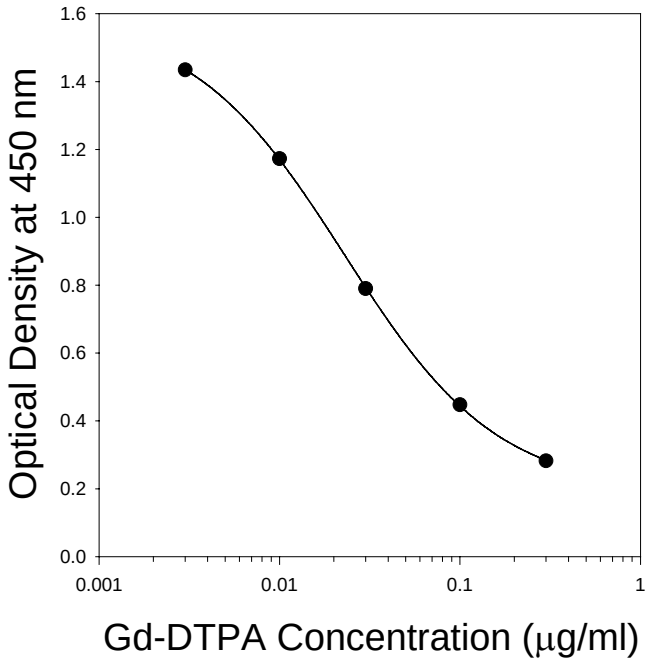
EXAMPLE (Typical Standard Curve Data)

Standards (µg/ml)	0.003	0.01	0.03	0.1	0.3
	OPTICAL DENSITIY (450 nm)				
	1.524	1.225	0.833	0.457	0.241
	1.490	1.200	0.829	0.457	0.248
	1.526	1.243	0.871	0.468	0.251
Average	**1.514**	**1.222**	**0.845**	**0.461**	**0.247**
B/Bo	**1.000**	**0.807**	**0.558**	**0.304**	**0.163**
STANDARD DEVIATION	**0.020**	**0.022**	**0.023**	**0.007**	**0.005**
% CV	**1.33**	**1.77**	**2.76**	**1.47**	**2.11**

EXAMPLE OF A STANDARD CURVE



Note: Do not use example values in place of standard curve determined at the time of the assay.

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B. SAMPLES

1. Record the absorbance at 450 nm for each specimen well.

2. Calculate and record the mean absorbance for each pair of specimen duplicates. Correct all values by subtracting the mean value for the blank from each specimen value.

3. Locate the corrected mean absorbance value, which corresponds to each specimen on the vertical axis, and follow a horizontal line intersecting the Standard Curve. At the point of intersection, read the Gd-DTPA concentration from the horizontal axis. For best results, use curve-fitting software that automatically interpolates this data *via* a 4-parameter curve fit and equation. The interpolated value will need to be multiplied by the dilution factor to obtain the actual Gd-DTPA concentration of the sample.

4. The measured values of Gd-DTPA in each sample can then be used to calculate the glomerular filtration rate using appropriate methods for the chosen protocol (please refer to Manual for Calculation of GFR in Research Subjects, Cat. No.: FIT-0007).

LIMITATIONS

1. FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES.
2. Since assay conditions may vary from assay to assay, a standard curve must be established for every run.
3. Since cross contamination between reagents will invalidate the test, disposable pipette tips should be used.
4. Reusable glassware must be washed and thoroughly rinsed of all detergent before use. Disposable flasks or glassware are preferred.
5. Thorough washing of the wells after 1.5-hour incubation is required:
    a. Completely aspirate well contents before dispensing fresh wash solution.
    b. Fill with wash solution to the top of the well for each wash cycle (approximately 350 µl).
    c. Do not allow wells to sit uncovered or dry for extended periods between steps.
6. This assay is not qualified for EDTA plasma.
7. The 96-well plate is NOT re-useable.
8. The instructions regarding well volumes for reagents cannot be altered.

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PERFORMANCE CHARACTERISTICS

A. SENSITIVITY

The detection limit of Gd-DTPA is approximately 0.5 picograms.

B. PRECISION

Intra-assay precision was determined by choosing two Gd-DTPA concentrations that fall on the active range of the curve and assaying 12 replicates. The chosen Gd-DTPA concentrations were 0.0635 and 0.0198 µg/ml, respectively.

	Concentration Gd-DTPA (µg/ml)	
	0.0635	0.0198
	OPTICAL DENSITY	(450 nm)
	0.622	1.049
	0.626	1.060
	0.640	1.046
	0.622	1.028
	0.621	1.034
	0.610	1.053
	0.617	1.014
	0.633	1.063
	0.633	1.077
	0.619	1.051
	0.611	1.055
	0.599	1.049
Average:	**0.621**	**1.048**
Standard Deviation:	**0.011**	**0.017**
% CV:	**1.808**	**1.592**

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