Catalog Number: FIT-0115 96-well Test Kit

Functional Immunoassay Technology (FIT™) Molecular Imaging and Tracking Technology (MITT)

FIT-MITT™ Kit (Gd-DTPA)

Patents Pending

An enzyme immunoassay test kit for the determination of gadolinium-DTPA (Gd-DTPA) in collected samples of interest

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

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BioPhysics Assay Laboratory (BioPAL), Inc.
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PROPRIETARY NAME

Functional Immunoassay Technology (FIT™)
FIT-MITT™ Kit
BioPhysics Assay Laboratory (BioPAL), Inc.
Catalog Number: FIT-0115, 96-Well Test Kit

INTENDED USE

The FIT-MITT assay is an enzyme immunoassay used for the determination of Gd-DTPA conjugates in collected samples. As a result, researchers can track and quantify compounds that have been labeled with Gd-DTPA via BioPAL's Gd-DTPA protein labeling kits.

THE FIT-MITT™ 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)**
   Optional: 1 mg/ml Concentrate should be diluted using Standard and Sample Diluent to appropriate concentrations. This reagent can be used to quantify total Gd-DTPA content. However, researchers MUST develop a standard curve using their Gd-DTPA labeled compound.
   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-MITT (Gd-DTPA) Kit Manual (1)**
   Catalog # FIT-0107
9. **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. Researcher 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. 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 (Speed setting: 500 rpm)

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

The FIT-MITT ELISA can be applied to a wide range of biological and non-biological samples for the quantification of Gd-DTPA conjugates. Researchers will need to develop and validate sample preparation procedures for their unique application. For example, a venous blood sample can be 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. 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 according to procedure and without interruption.
7. Interruption of the procedure or changing the order of reagent addition will invalidate kit performance.

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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

To quantify your Gd-DTPA labeled protein or compound, researcher MUST develop a standard curve using your labeled compound. The above range provided for Gd-DTPA can serve as a guide.

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 samples.

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 sample 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.**

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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. NOTE: Due to differences between models and manufactures, adjustment of the orbital shaker speed may need to be optimized.

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-labeled compound 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 labeled compound 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-labeled compound 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-labeled compound in the sample.

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Scheme 1: Example Protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	blank	blank	Sample 1	Sample 1								
B	Your Compound **0.003**	Your Compound **0.003**	Sample 2	Sample 2								
C	Your Compound **0.01**	Your Compound **0.01**	Sample 3	Sample 3								
D	Your Compound **0.03**	Your Compound **0.03**	Sample 4	Sample 4								
E	Your Compound **0.1**	Your Compound **0.1**	Sample 5	Sample 5								
F	Your Compound **0.3**	Your Compound **0.3**	Sample 6	Sample 6								
G	Low Control	Low Control										
H	High Control	High Control										

RESULTS

Gd-DTPA-labeled compound 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 Gd-DTPA-labeled compound 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 sample 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 sample on the vertical axis, and follow a horizontal line intersecting the Standard Curve. At the point of intersection, read the Gd-DTPA-labeled compound 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-labeled compound concentration of the sample.

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 conjugate and assay 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 or EDTA containing samples.

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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