Instruction Manual No. M-PG-RIG-50

PureGel Rabbit IgG- Direct ELISA for the quantitative measurement of Rabbit IgG bound to agarose beads

Cat. #PG-RIG-50; 50 Tests

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544

Toll Free (800) 786-5777

Email: service@4adi.com

Web Site: www.4adi.com

Kit Components, 96 tests	Cat #
PureGel Rabbit IgG Standard. **A** (0 ng/ml), 0.65 ml	PGRIG-51A
PureGel Rabbit IgG Standard. **B** (10 ng/ml), 0.65 ml	PGRIG -51C
PureGel Rabbit IgG Standard. **C** (100 ng/ml), 0.65 ml	PGRIG -51D
PureGel Rabbit IgG Standard. **D** (300 ng/ml, 0.65 ml	PGRIG -51E
PureGel Rabbit IgG Standard. **E** (1000 ng/ml), 0.65 ml	PGRIG -51F
Note: All Standards are made in Agarose suspension. Shake well and mix before use.	
PureGel Sample Diluent, pink solution, 15 ml (**mix the contents prior to use)**	PGRIG-52
Anti-Rabbit IgG-HRP Conjugate, 90 ul (**100X**),	PGRIG-53
HRP Conjugate Diluent, 1 ml (**20X**)	SD-20T
Agarose stabilizing solution (**mix the contents prior to use)**	AGR-S
Wash buffer (100X), 10 ml; dilute 1:100 with distilled water	WB-100
HRP substrate, Solution, 12 ml	80091
Stop solution, 12 ml	80101
ELISA Strip Plate (8x12 or 96 wells)	PG-P1
PureGel Assay Tubes, 50	PGRIGT-50
Qtips, 50	PGRIGQ-50
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Proteins of interest can be purified from crude antiserum or other complex mixtures by a variety of methods. Affinity chromatography is a simple and specific method for separating one type of protein from others. Affinity chromatography typically involves the interaction of a stationary antigen or ligand (solid phase) with antibody in solution such as antiserum (mobile phase). The antigens or ligands are chemically immobilized to a solid support, most commonly agarose beads, so that when the mixture is passed over the column, the antibodies will become bound to the antigen coupled to the agarose while the rest of the non-specific components are washed away. After washing, the bound antibody is then eluted from the support, resulting in the purification of the antibody.

The elution buffer used can have significant impacts on the efficiency of dissociation and specific activity of the purified antibodies. The most common elution method used is typically a low pH method using pH 2.5-3, 0.1M glycine. Antibodies can also be purified though altering the ionic strength with buffers such as 4M magnesium chloride, 2.5M sodium iodide, or 5M NaCl. The benefit of ionic disruption are retaining protein functionality and easy column regeneration. Ionic disruption is considered the least harsh method of elution and may not elute out higher affinity antibodies. Harsh methods of elution such as a 6M Guanidine HCL, 8M urea, or 1% SDS will typically elute most of the antibody although it will denature the antibody, limiting its ability to be used in downstream applications.

ADI's PureGel kit is the world's first and only kit designed to quantitate the amount of antibody captured to antigen-coupled agarose beads before and after elution to allow for quantitative comparisons on the efficiency of various elution buffers. It is a simple, rapid, and sensitive test that requires no extraction or harsh dissociation of the antigens from the agarose.

PRINCIPLE OF THE TEST

PureGel Rabbit IgG ELISA kit is based on direct binding of an anti-Rabbit IgG -HRP conjugate to Rabbit IgG bound to agarose. After a washing step, chromogenic substrate is added and color is developed. The enzymatic reaction (color) is directly proportional to the amount of Rabbit IgG present on the beads. Adding a stopping solution terminates the reaction. The absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of Rabbit IgG in the samples are calculated based off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipette (5-1000 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader. Table top microfuge

PRECAUTIONS AND SAFETY INSTRUCTIONS

ADI's PureGel ELISA kit is intended for *in vitro research* use only. Standards, HRP diluent, and HRP conjugate contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website:

http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf

SAMPLE COLLECTION AND HANDLING

This kit is designed to measure Rabbit IgG bound to antigen-agarose beads. Do not add azide or other preservatives to the gel. Do not freeze the gel. This kit is not suitable for the measurement of Rabbit IgG in solution (not bound to agarose). ADI has other kits to measure Rabbit IgG in serum or plasma or biological buffers.

REAGENT PREPARATION FOR THE ASSAY

Dilute wash buffer (1:100) with distilled water (10 ml WB-100 in 990 ml). Store at 2-8ºC.

Prepare 1X Sample Diluent: Dilute SD-20T 1:20 with water. Store at Store at 2-8ºC

Dilute enzyme conjugate 1:100 (*e.g.,*10 ul of HRP-conjugate in 1X 990 ul SD-20T). You will need 150 ul of 1X conjugate per sample. It is recommended to prepare more than needed to avoid the formation of bubbles during pipetting. Return unused stock to 2-8ºC. Do not store 1X working solutions beyond the date the assay was performed.

SAMPLE PREPARATION AND EXPECTED VALUES

We suggest to first try a 1:100 dilution of the gel. Extensive in-house testing has shown that concentrations on the agarose detected typically range from 5-20% of the eluted antibody concentration when using an elution buffer such as pH 2.5 0.1M Glycine. Due to the size of the beads, a standard 200 ul tip may not be able to suck in the gel. A small portion of the tip may need to be cut off or a wide mouth pipette tip should be used instead. For greater accuracy, it is recommended to make an initial 1:10 stock (*e.g.*, 5 ul of gel into 45 ul of PureGel diluent). This stock can be used to prepare further dilutions if necessary. Dilutions of all agarose beads must be done in the supplied PureGel diluent only. Do not use any other diluents.

STORAGE AND STABILITY

The kit contents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is at least 6 months from the date of shipping under appropriate storage conditions..

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM* TEMPERATURE BEFORE USE*).* Important: If you have not used this kit before, we recommend to **run the standards alone** to get familiar with the test and not run the risk of making mistakes and losing samples or the whole kit.

We recommend all standards and samples be tested in duplicate. Remove the required number of supplied 1.5 ml assay tubes, corresponding the number of tests. Store unused tubes and strips in the supplied bag

1. Label the required number of 1.5 ml assay tubes and collect the required number of blank ELISA strips as well. If necessary break off the wells and arrange them on the well holder. The ELISA wells are only used to read the A_{450} values of the samples at the end of the assay. It is possible to use ELISA strips or ELISA plates from other suppliers as well.

2. Dilute Agarose samples as necessary in PureGel diluent only (pink solution). See 'Sample preparation and expected values' section (Page 2) for suggested dilutions. Note: The gels will settle at the bottom during storage. It should be gently mixed for 5-10 seconds before use. .

3. PureGel stabilizer solution (green) is a bit turbid or may have a cloudy appearance. It should be gently mixed by manual shaking or inversion the bottle for 5-10 seconds prior to every use. **Pipet 300 ul of stabilizer solution** to an appropriate number of labeled 1.5 ml conical assay tubes supplied in the kit.

4. **Do not dilute the standards**. Gently mix the standards by vortexing for 5-10 seconds. **Dispense 100 ul of the standards and samples** in duplicate into the tubes containing **300-ul stabilizing solution**. **Close the caps and mix the contents by vortexing for 2-3 seconds; incubate for 30 mins at room temperature**.

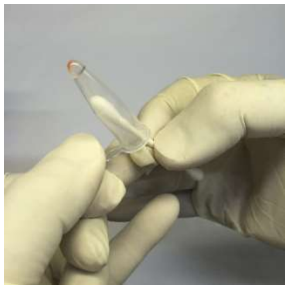
5. Centrifuge the tubes for 60 seconds in a microfuge at 2,000Xg at room temperature. Time and speed may have to be adjusted based on each centrifuge. **Note**: The pinkish/brownish small pellet of Agarose gel at the bottom of all tubes. Carefully invert the tube and discard the supernatant in a waste container. Keep the tubes inverted and tap over a paper towel a few times to remove any remaining solution.

   You must not disturb the gel pellet or discard it as it contains the agarose antigen-antibody complex. The pellet will remain at the bottom of the tube during the process. Return all tubes to the tube holder.

6. Add **150 ul of 1X antibody-HRP conjugate** to each tube**.** Vortex each tube 3-5 seconds to mix the pellet with the conjugate solution. **Note**: The gel pellet must have a uniform suspension, failure to achieve a uniform suspension will lead to lower OD_{450} or high variance in duplicates.. After mixing, **incubate all tubes for 60 minutes** at room temperature.

7. Centrifuge the tubes for 60 secs in a microfuge at 2,000xg at room temperature as in step 5. **Note** the small pellet of Agarose gel at the bottom of each tube. Carefully invert the tube and remove the conjugate solution as in step 5. Wash the pellet by adding **300 ul** of 1x wash buffer into all tubes. Vortex to mix and resuspend the pellet to make uniform suspension. Repeat the pellet wash 4 times more for a total of 5-washes. **Note**: After each wash, the tubes must be tapped over paper towels to remove excess liquid. Failure to wash properly will produce higher non-specific binding.

8. After the last wash, remove all liquid from the tube and tap over fresh paper towels. Observe each tube for any liquid or droplets sticking on the tube walls. Remove traces of HRP-conjugate from the tube walls using supplied Q-tips. Failure to remove the wash solution will result in higher blanks. Do not disturb the pellet or touch it with the Qtip.



9. **Pipette 150 ul of TMB substrate solution per tube.** Close the cap and vortex each tube for 3-5 seconds to make sure that the gel pellet is completely dispersed. Failure to perform this step properly will give spurious readings or irregular duplicates. **Incubate for 15 minutes at room temperature**. Incubation time may be changed $\pm$ 5 minutes to obtain a high standard of OD_{450} 2.00-3.00 if necessary. A blue color should develop in high standards.

10. **Stop the reaction by adding 150 ul of 'Stop solution'** to all tubes. Mix gently for 3-5 seconds to ensure even color distribution. The blue color will turn yellow in positive samples

11. **Centrifuge the tubes for 60-seconds at 2,000xg**. **Carefully take 200 ul of the supernatant using a pipette and transfer to the ELISA strip wells for measuring the absorbance**.

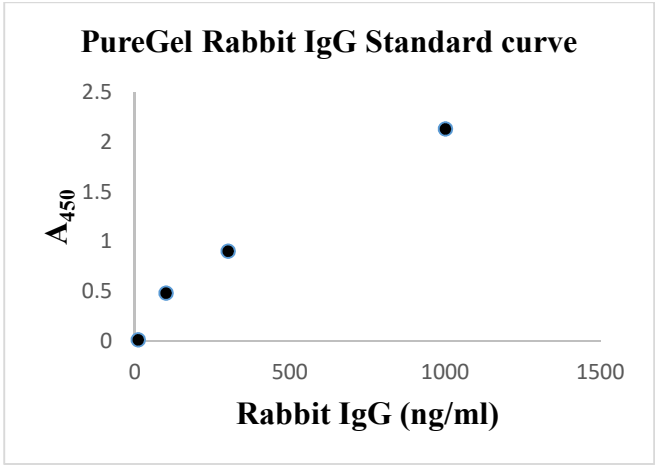
12. Measure the absorbance at 450 nm with 630 nm as a reference filter if available using an ELISA reader. The reaction is stable for at least 30 minutes after stopping.

WORKSHEET OF TYPICAL ASSAY

Wells	Standards/samples	Mean $A_{450\ nm}$	Net $A_{450\ nm}$
A1, A2	**PureGel Standard A** (0 ng/ml)	0.144	0
B1, B2	**PureGel Standard B** (10 ng/ml)	0.173	0.029
C1, C2	**PureGel Standard C** (100 ng/ml)	0.628	0.484
D1, D2	**PureGel Standard D** (300 ng/ml)	1.047	0.903
E1, E2	**PureGel Standard E** (1000 ng/ml)	2.275	2.131
F1, F2	**Sample 1** (diluted 1:100)	1.06	0.916

Sample 1 concentration: 326.7 ng/mlX100= 32.67 ug/ml

NOTE: This data is for demonstration purpose only. A complete standard curve must be run in every assay to determine sample concentrations. Standard A should not be used in the curve, only to subtract background from standard and sample values



A typical assay standard curve (do not use these values for calculating sample values)

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards and samples. Draw the standard curve on a graph paper by plotting net absorbance values of standards against appropriate Rabbit IgG concentrations. Read off the Rabbit IgG concentrations of the samples directly from the standard curve. If samples were diluted, then the concentration should be multiplied by the dilution factor.

If an ELISA reader software is being used, we recommend 4-paramter or 5-parameter curve.

PERFORMANCE CHARACTERISTICS

Detection limit- Based on replicate determinations of the zero standard, the minimum Rabbit IgG concentration detectable using this assay is ~5 ng/ml of Rabbit IgG. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Specificity

PureGel Rabbit IgG Direct ELISA has been tested against agarose gels coupled with peptides bound with Goat IgG, Mouse IgG, Sheep IgG, and Protein A/G coupled antibodies. The assay shows no reactivity against antibodies against other species.

This kit is not suitable for measuring Rabbit IgG in solution. ADI has developed other ELISA kits for the measurement of Rabbit IgG.

Precision

Samples (n=5) were run on the same lot to determine in intra-assay variability. Samples (n=5) were run across multiple days on various lots to determine inter-assay variability

	High	Medium	Low
Inter-assay	2.52%	3.69%	2.79%
Intra-assay	2.49%	8.2%	7.73%

Suggestions for good performance with the PureGel Rabbit IgG ELISA

The PureGel ELISA is an unusual test because the antigen-antibody reaction is being performed directly on the agarose that is in a gel suspension. Therefore, it is very important to take a uniform amount of the sample gel and dilute it in PureGel diluent (pink) in order to avoid loss of the small gel particle during prolonged processing of the test. User's must get a good understanding of the protocol and the role of each step. The PureGel ELISA differs from a regular ELISA due to the nature of the samples (Agarose gels). It very critical to be patient during the manual wash process and remove traces of the wash solutions without losing the sample (gel pellet). Some common issues observed:

1. **High standards give low values**- 1) Generally due to the loss of the standard gel pellet during the assay. 2) Not mixing the standards before taking the samples. 3) Antibody-HRP used at a lower concentration or higher dilution than the recommended 1:100.
2. **Standards or duplicates show high variations-** Generally one more of the issues seen in example 1
3. **Agarose pellet too tight and does not resuspend easily –** Gel Suspension may have been spun too long or too fast. We recommend 2,000xg for 60 seconds at each step. Lower the speed or time if the pellet is too tight.
4. **Agarose gel pellet too loose –** Gel suspension may have been spun too short or too slow. We recommend 2,000xg for 60 secs at each step. Increase the speed or time of centrifugation if a loose pellet is observed
5. It is recommended to run just the standards and a few samples to get familiar with the protocol before running too many samples to avoid loss of the reagents or kit.

ELISA kits available from ADI (see details at the web site)

Catalog#	Product Description
VAC-DTX-50	VacciGel™ Direct ELISA for the measurement of Diphtheria Toxoid in Vaccines formulated in Alum
VAC-DTX-210	Diphtheria Toxoid/Toxin (DTX) ELISA for the measurement DTX in biological buffer
VAC-HBS-50	VacciGel™ Direct ELISA for the measurement of Hepatitis B Vaccine (HBsAg) formulated in Alum
VAC-HCG-50	VacciGel™ Direct ELISA for the measurement of HCG (contamination) in Vaccines formulated in Alum
VAC-PTX-50	VacciGel™ Direct ELISA for the measurement of Pertussis Toxoid in Vaccines formulated in Alum
VAC-PTX-410,	Pertussis Toxoid/Toxin (PTX) ELISA for the measurement PTX in biological buffer
VAC-TTX-50	VacciGel™ Direct ELISA for the measurement of Tetanus Toxoid in Vaccines formulated in Alum
VAC-TTX-310	Tetanus Toxoid/Toxin (TTX) ELISA for the measurement TTX in biological buffer
6520	Rabbit IgG ELISA Kit, 96 tests, Quantitative
20009-1	Rabbit IgG, purified (serum non-immune, isotype control)
20009-1-100	Rabbit IgG (negative control for flow cytometry)
20009-1-200	Rabbit IgG Control Sera
20009-1-CH	Rabbit IgG (non-immune, isotype control, CHIP/IP grade)
20009-1-FAB	Rabbit IgG Fab fragment, purified
20009-1-FAB2	Rabbit IgG F(ab')2 fragment, purified
20009-1-FAB-B	Rabbit IgG Fab-Biotin Conjugate, purified
20009-1-FAB-F	Rabbit IgG Fab-FITC Conjugate, purified
20009-1-FAB-HP	Rabbit IgG Fab-HRP Conjugate, purified
20009-1-FC	Rabbit IgG (Fc) fragment, purified