ab133031 – 15-deoxy-delta^{12,14}-PGJ_2 ELISA Kit

Instructions for Use

For quantitative detection of 15-deoxy-delta^{12,14}-PGJ_2 in tissue culture media, saliva, urine, and plasma from a wide range of species.

This product is for research use only and is not intended for diagnostic use.

Version 6a Last Updated 14 August 2025

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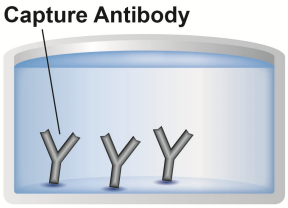
1. BACKGROUND

Abcam's 15-deoxy-delta^{12,14}-PGJ_2 *in vitro* competitive ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of 15-deoxy-delta^{12,14}-PGJ_2 in tissue culture media, saliva, urine and plasma from a wide range of species.

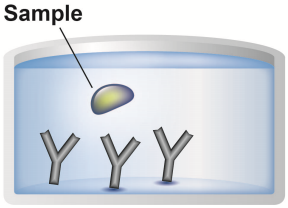
A goat anti-rabbit IgG antibody has been precoated onto 96-well plates. Standards or test samples are added to the wells, along with an alkaline phosphatase (AP) conjugated-15-deoxy-delta^{12,14}-PGJ_2 antigen and a polyclonal rabbit antibody specific to 15-deoxy-delta^{12,14}-PGJ_2. After incubation the excess reagents are washed away. pNpp substrate is added and after a short incubation the enzyme reaction is stopped and the yellow color generated is read at 405 nm. The intensity of the yellow coloration is inversely proportional to the amount of 15-deoxy-delta^{12,14}-PGJ_2 captured in the plate.

15-deoxy-delta^{12,14}-PGJ_2 (15-d-PGJ_2) is one of the ultimate dehydration products of PGD_2. PGD_2 is formed from PGH_2 which itself is synthesized from arachidonic acid by the enzyme prostaglandin synthase. In the aqueous solution PGD2 forms PGJ_2. Albumin or other serum proteins present in the sample will convert PGD_2 to the isomeric compound, delta^{12}-PGJ_2. In males about 152 ng of delta^{12}-PGJ_2 is excreted in urine per day, and in women about half this amount is excreted. 15-d-PGJ_2 has been shown as an inducer of adipogenesis and an activator of the γ isoform of the peroxisome proliferation activated receptor (PPAR-gamma).

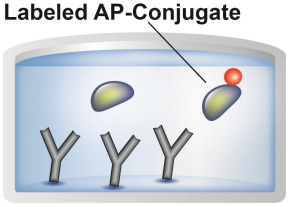
2. ASSAY SUMMARY



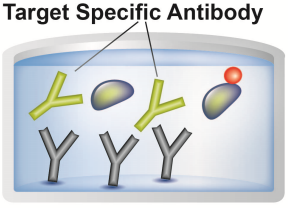
Prepare all reagents and samples as instructed.



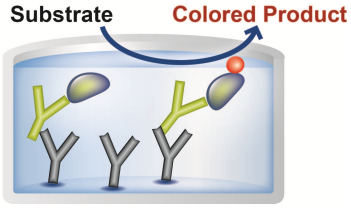
Add standards and samples to appropriate wells.



Add prepared labeled AP-conjugate to appropriate wells.



Add 15-deoxy-delta^{12,14}-PGJ_2 antibody to appropriate wells. Incubate at room temperature.



Add pNpp substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- Some kit components contain azide, which may react with lead or copper plumbing. When disposing of reagents always flush with large volumes of water to prevent azide build-up
- Stop Solution is a solution of trisodium phosphate. This solution is caustic; care should be taken in use
- The activity of the alkaline phosphatase conjugate is dependent on the presence of Mg^{2+} and Zn^{2+} ions. The activity of the conjugate is affected by concentrations of chelators (>10 mM) such as EDTA and EGTA
- We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results
- The 15-d-PGJ_2 Standard provided is supplied in ethanolic buffer at a pH optimized to maintain 1515-d-PGJ_2 integrity. Care should be taken handling this material because of the known and unknown effects of 15-d-PGJ_2.

4. STORAGE AND STABILITY

Store kit at 4°C immediately upon receipt, apart from the Alkaline Phosphatase Conjugate and Standard, which should be stored at -20°C. Avoid multiple freeze-thaw cycles.

Refer to list of materials supplied for storage conditions of individual components.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Goat anti-rabbit IgG Microplate (12 x 8 wells)	96 Wells	2-8°C
15-d-PGJ_2 Alkaline Phosphatase Conjugate	5 mL	-20°C
Anti-15-d-PGJ_2 Antibody	5 mL	2-8°C
15-d-PGJ_2 Standard	500 µL	-20°C
Assay Buffer	27 mL	2-8°C
20X Wash Buffer Concentrate	27 mL	2-8°C
pNpp Substrate	20 mL	2-8°C
Stop Solution	5 mL	2-8°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Standard microplate reader - capable of reading at 405 nm, preferably with correction between 570 and 590 nm
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes
- Microplate Shaker
- Absorbent paper for blotting
- 200 mg C_{18} Reverse Phase Extraction Columns (only required for extraction of samples containing low levels of 15-d-PGJ_2)
- 2M hydrochloric acid (only required for extraction of samples containing low levels of (15-d-PGJ_2)
- Deionized water
- 37°C incubator
- Ethanol
- Hexane
- Ethyl Acetate

7. LIMITATIONS

- Assay kit intended for research use only, not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

8. TECHNICAL HINTS

- Standards can be made up in either glass or plastic tubes
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent
- Pipette standards and samples to the bottom of the wells
- Add the reagents to the side of the well to avoid contamination
- This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided
- Care must be taken to minimize contamination by endogenous alkaline phosphatase. Contaminating alkaline phosphatase activity, especially in the substrate solution, may lead to high blanks. Care should be taken not to touch pipet tips and other items that are used in the assay with bare hands
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.

9.1 15-d-PGJ$_2$ Alkaline Phosphatase Conjugate

Allow the 15-d-PGJ$_2$ Alkaline Phosphatase Conjugate to equilibrate to room temperature. Any unused conjugate should be aliquoted and re-frozen at or below -20°C.

9.2 Conjugate 1:10 Dilution for Total Activity Measurement

Prepare the Conjugate 1:10 Dilution by diluting 50 µL of the supplied conjugate with 450 µL of Assay Buffer. The dilution should be used within 3 hours of preparation. This 1:10 dilution is intended for use in the Total Activity wells only.

9.3 1X Wash Buffer

Prepare the 1X Wash Buffer by diluting 5 mL of the 20X Wash Buffer Concentrate in 95 mL of deionized water. Mix thoroughly and gently.

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Diluted standards should be used within 60 minutes of preparation.

10.1 Allow the 1,000,000 pg/mL 15-d-PGJ2 Stock Standard solution to warm to room temperature. The standard solution should be stored at -20°C. Avoid repeated freeze-thaw cycles.

10.2 Label six tubes with numbers 1 – 6 and one with B_O.

10.3 Prepare a 200,000 pg/mL **Standard 1** by adding 200 µL of the 1,000,000 pg/mL Stock Standard to 800 µL of the appropriate diluent into tube 1. Mix thoroughly and gently.

10.4 Add 750 µL of the appropriate sample diluent into tubes numbers 2- 7.

10.5 Prepare **Standard 2** by transferring 250 µL from Standard 1 to tube 2. Mix thoroughly and gently.

10.6 Prepare **Standard 3** by transferring 250 µL from Standard 2 to tube 3. Mix thoroughly and gently.

10.7 Using the table below as a guide, repeat for tubes 4 through 6.

Standard	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Standard	200	800	1,000,000	200,000
2	Standard 1	250	750	200,000	50,000
3	Standard 2	250	750	50,000	12,500
4	Standard 3	250	750	12,500	3,125
5	Standard 4	250	750	3,125	781
6	Standard 5	250	750	781	195
7	None	-	750	-	0


1
2
3
4
5
6

11. SAMPLE COLLECTION AND STORAGE

- This kit is compatible with 15-d-PGJ_2 samples in a wide range of matrices. Samples diluted sufficiently into Assay Buffer can be read directly from the standard curve. However, the end user must verify that the recommended dilutions are appropriate for their samples.
- Samples containing rabbit IgG may interfere with the assay.
- Some samples normally have very low levels of 15-d-PGJ_2 present and extraction may be necessary for accurate measurement. A suitable extraction procedure is outlined below:

11.1 Materials Needed

11.1.1 15-d-PGJ_2 Standard to allow extraction efficiency to be accurately determined.

11.1.2 2M hydrochloric acid, deionized water, ethanol, hexane and ethyl acetate.

11.1.3 200 mg C18 Reverse Phase Extraction Columns.

11.2 Procedure

11.2.1 Acidify the plasma, urine or tissue homogenate by addition of 2M HCl to pH of 3.5. Approximately 50 µL of HCl will be needed per mL of plasma. Allow to sit at 4 °C for 15 minutes. Centrifuge samples in a microcentrifuge for 2 minutes to remove any precipitate.

11.2.2 Prepare the C18 reverse phase column by washing with 10 mL of ethanol followed by 10 mL of deionized water.

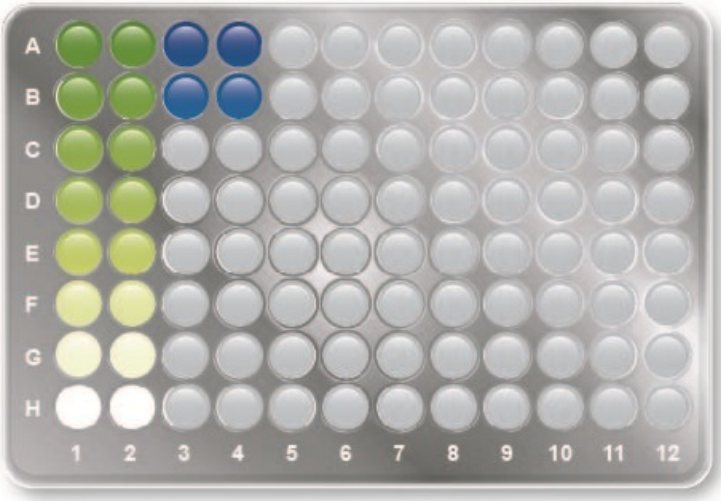
11.2.3 Apply the sample under a slight positive pressure to obtain a flow rate of about 0.5 mL/minute. Wash the column with 10 mL of water, followed by 10 mL of 15% ethanol, and finally 10 mL hexane. Elute the sample from the column by addition of 10 mL ethyl acetate.

11.2.4 If analysis is to be carried out immediately, evaporate samples under a stream of nitrogen. Add at least 250 µL of Assay Buffer to the dried samples. Vortex well, then allow to sit 5 minutes at room temperature. Repeat twice more. If analysis is to be delayed, store samples as the eluted ethyl acetate solutions at -80 °C until the immunoassay is to be run. Evaporate the organic solvent under a stream of nitrogen prior to running assay and reconstitute as above.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

Recommended plate layout



A1-2 - Standard #1
B1-2 - Standard #2
C1-2 - Standard #3
D1-2 - Standard #4
E1-2 - Standard #5
F1-2 - Standard #6
G1-2 - Standard #7 (Bo)
H1-2 - Blank Wells

A3-4 - Total Activity Wells (TA)
B3-4 - Non-Specific Binding Wells (NSB)

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use**
- **It is recommended to assay all standards, controls and samples in duplicate**
- **Refer to the recommended plate layout in Section 12 before proceeding with the assay**

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Add 100 µL appropriate standard diluent (Assay Buffer or tissue culture media) into the NSB and Bo wells.

13.3 Add 100 µL of prepared standards and diluted samples to appropriate wells.

13.4 Add 50 µL of Assay Buffer into the NSB wells.

13.5 Add 50 µL of 15-d-PGJ_2 Alkaline Phosphatase Conjugate into each well, except the TA and Blank wells.

13.6 Add 50 µL of 15-d-PGJ_2 antibody into each well, except the TA, Blank and NSB wells.

Note: Every well used should be Green in color except the NSB wells which should be Blue. The Blank and TA wells are empty at this point and have no color.

13.7 Incubate the plate at room temperature on a plate shaker for 2 hours at ~500 rpm. The plate may be covered with the plate sealer provided.

13.8 Empty the contents of the wells and wash by adding 400 µL of 1X Wash Buffer to every well. Repeat the wash 2 more times for a total of 3 washes.

13.9 After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

13.10 Add 5 µL of the 1:10 diluted 15-d-PGJ_2 Alkaline Phosphatase Conjugate to the TA wells.

13.11 Add 200 µL of the pNpp Substrate solution to every well. Incubate at 37ºC for 3 hours without shaking.

13.12 Add 50 µL Stop Solution into each well.

13.13 Blank the plate reader against the blank wells, read the O.D. absorbance at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the blank wells, manually subtract the mean optical density of the blank wells from all readings.

14. CALCULATIONS

A four-parameter algorithm (4PL) provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4-parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted.

1. Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average NSB OD from the average OD bound:

   Average Net OD = Average Bound OD - Average NSB OD

2. Calculate the binding of each pair of standard wells as a percentage of the maximum binding wells (Bo), using the following formula:

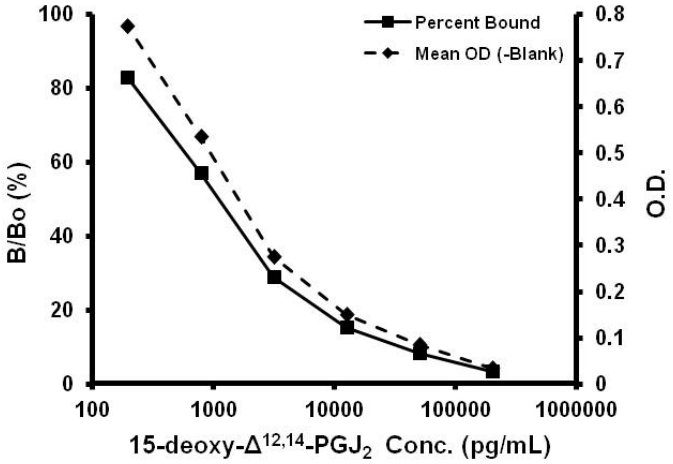
   Percent Bound = (Net OD/ Net Bo OD) x 100

3. Plot both the Percent Bound and the Net OD versus Concentration of 15-d-PGJ_2 for the standards. Sample concentrations may be calculated off of Net OD values using the desired curve fitting

- Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Sample	Mean OD (- Blank)	% Bound	15-d-PGJ_2 (pg/mL)
Blank	(0.134)		
TA	2.696		
NSB	0.009	0	
B_O	0.932	100	0
Standard 1	0.034	3.4	200,000
Standard 2	0.086	8.3	50,000
Standard 3	0.151	15.4	12,500
Standard 4	0.276	28.9	3,125
Standard 5	0.535	57	781
Standard 6	0.775	83	195
Unknown 1	0.624	66.6	486
Unknown 2	0.102	10.1	23,638

TYPICAL QUALITY CONTROL PARAMETERS

Total Activity Added	= 2.696 x 10 x 10 = 269.6
%Bo/TA	= 0.3%
Quality of Fit	= 0.9997 (Calculated from 4 parameter logistic curve fit)
20% Intercept	= 7,636 pg/mL
50% Intercept	= 1,104 pg/mL
80% Intercept	= 247 pg/mL

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The sensitivity, minimum detectable dose of 15-d-PGJ_2using this Abcam ELISA kit was found to be 36.8 pg/mL. This was determined by the average optical density of the 0 pg/mL Standard and comparing to the average optical density for Standard 6. The detection limit was determined as the concentration of 15-d-PGJ_2 measured at two standard deviations from the zero along the standard curve.

SAMPLE RECOVERY –

Recovery was determined by 15-d-PGJ_2 into biological fluids. Mean recoveries are as follows:

Sample Type	Average %Recovery	Recommended Dilution
Tissue Culture Media	97.4	1:2
Human Saliva	94.4	None
Human Urine	113.9	1:16
Porcine Plasma	105.0	≥1:4

LINEARITY OF DILUTION –

A sample containing 13,011 pg/mL 15-d-PGJ_2 was diluted 4 times 1:2 in the kit Assay Buffer and measured in the assay. The data was plotted graphically as actual 15-d-PGJ_2 concentration versus measured 15-d-PGJ_2 concentration.

The line obtained had a slope of 0.974 and a correlation coefficient of 0.998.

PRECISION –

	15-d-PGJ_2 (pg/mL)	**Intra-Assay %CV**
Low	1,136	5.6
Medium	3,062	5.7
High	4,394	7.4

	15-d-PGJ_2 (pg/mL)	**Inter-Assay %CV**
Low	1,195	15.7
Medium	4,406	13.0
High	7,219	14.5

17. ASSAY SPECIFICITY

This kit detects both endogenous and recombinant 15-d-PGJ_2.

CROSS REACTIVITY –

The cross reaction of the antibody calculated at 50% is:

15-deoxy-delta^{12,14}-PGJ_2	100 %
PGJ_2	49.2%
delta^{12}-PGJ_2	5.99%
PGD_2	4.92%
Arachidonic Acid	0.03 %
PGE_2	<0.01%
PGF_2 alpha	<0.01 %
9α,11β-PGF_2 alpha	<0.01 %
Thromboxane B_2	<0.01 %
2-Arachidonoylglycerol	<0.01 %
Anandamide	<0.01 %

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

19. NOTES

Technical Support

For technical or commercial enquiries please go to:

www.abcam.com/contact (US)

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)