Performance characteristics, continued

Colored interferents

Three food color preparations (FD&C Red No. 40 and Red No. 3; Yellow No. 5; Blue No. 1) were used to assess the effect of colored APIs on the measured palladium concentration in the assay.

Sample	1:800 dye dilution		1:3,200 dye dilution	
	Pd conc. (nM)	% Interference	Pd conc. (nM)	% Interference
Pd	76.2	—	76.2	—
Pd + red dye	61.4	19.4	70.9	7.0
Pd + yellow dye	63.4	16.8	79.7	-4.6
Pd + blue dye	66.5	12.7	76.9	-0.9

Linearity of dilution

Five Pd catalysts dissolved in DMSO at 1mg/mL and dilutions of 20 and 5 nM Pd in Sample Diluent were run in the assay alongside $PdCl_2$ standards. The obtained values were evaluated for linearity off the lowest concentration (5 nM) and for percent recovery compared to control values.

Catalyst	Nominal conc. (nM)	Observed Conc (nM)	% Linearity	Observed control conc. (nM)	% Recovery
1	20	16.81	94.1	19.54	86.0
2	20	16.17	99.8	—	80.9
3	20	16.67	107.4	18.56	89.8
4	20	13.80	110.8	19.97	69.1
5	20	17.87	91.8	19.39	92.2

Catalyst 1 = Allylpalladium(II) chloride dimer
Catalyst 2 = Bis-(dibenzylideneacetone)palladium(0)
Catalyst 3 = Bis(triphenylphosphine)palladium(II) dichloride
Catalyst 4 = 2-(2'-Di-tert-butylphosphine) biphenylpalladium(II) acetate
Catalyst 5 = Palladium(II) acetate

API interaction tests

Five chemical compounds that bind extremely tightly to palladium catalysts that could be representative of structures found in APIs were dissolved in DMSO. Each compound was spiked with the Pd catalysts tested for recovery and the $PdCl_2$ standards at concentrations equivalent to 10 ppm Pd contamination.

Each compound without added palladium catalysts was run as a compound control. Each catalyst spiked into DMSO without a compound was run as catalyst controls.

Multiple dilutions of each of the solutions were made into Sample Diluent and run in the assay. No signal was detected for compound only controls.

Conc. catalyst or standard	Average % recovery				
	A	B	C	D	E
1 ≃ 10 ppm	101.6	78.7	54.6	61.3	ND
2 ≃ 10 ppm	93.8	67.6	36.7	73.4	ND
3 ≃ 10 ppm	155.8	58.8	ND	90.2	ND
4 ≃ 10 ppm	103.6	60.9	ND	48.7	75.6
5 ≃ 10 ppm	99.5	67.4	ND	75.0	ND
$PdCl_2$ Standard	107.4	81.2	ND	89.1	ND

ND = Not detectable

Compound A = 2-methyl-2- oxazoline
Compound B = thiazole
Compound C = 4,4'-diphenyl-2,2'-dipyridyl
Compound D = 8-aminoquinoline
Compound E = thiazolidine-2- carboxylic acid

Specificity

The Palladium Detection Reagent is selective for palladium and platinum over other metal ions.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

REF Catalog Number | LOT Batch code | Temperature limitation | Use by | Manufacturer | Consult instructions for use | Caution, consult accompanying documents

Manufacturer's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

The information in this guide is subject to change without notice.

Palladium API Fluorescent Detection Kit

Catalog Number EIAPDAPIF (96 tests)

Rev 3.0

For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Palladium API Fluorescent Detection Kit is a detection assay designed to rapidly determine the relative amounts of palladium present in active pharmaceutical ingredient (API) scavenging steps. The kit uses a proprietary non-fluorescent molecule that, under reducing conditions, is cleaved by palladium to yield a brightly fluorescent product (485 nm excitation, 520 nm emission).

Palladium (Pd) compounds are used in synthetic transformation in pharmaceutical processes, but palladium-catalyzed reactions present a problem in that the palladium can often be retained in the isolated product. A variety of methods are available for removing Pd from APIs, while standard methods of quantifying palladium in APIs are atomic absorption analysis, x-ray fluorescence, and plasma emission spectroscopy such as inductively-coupled plasma mass spectrometry (ICP-MS).

Contents and storage

Kit and components are shipped at 4°C. Upon receipt, store the kit at 4°C until the expiration date on the kit box. DO NOT FREEZE.

Components	Quantity
Palladium Standard; 2,000 nM palladium chloride in 1 M hydrochloric acid, **CAUSTIC**	100 µL
Black 96-well Plate	1 plate
Palladium Detection Reagent; palladium sensor solution in DMSO	3 mL
Sodium Borohydride Stock Solution; 2.5 M sodium borohydride in 10 M sodium hydroxide, **CAUSTIC**	400 µL
Borohydride Buffer; borate buffer containing stabilizers	5 mL
Sample Diluent; Tris buffer containing DMSO and stabilizers	60 mL

Materials required but not supplied

- Borosilicate glass tubes or vials
- N,N-dimethylformamide (DMF)
- Microtiter plate reader with software capable of measurement at or near 520 nm, with excitation at 485 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- (*Optional*) Microwave digestion apparatus with associated inert digestion cups, nitric acid, hydrochloric acid and/or 30% hydrogen peroxide

Procedural guidelines

Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

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

Sample dilution guidelines

This assay is designed to assess scavenging methods with samples in a variety of solvent systems: toluene, ethanol, acetonitrile, DMSO, DMF, N-methyl pyrrolidone (NMP), or dilute hydrochloric acid.

- API solutions should be made in borosilicate containers.
- If using ethanol or toluene, prefill the tip several times to ensure solvent vapor fills the space above the tip to ensure accurate delivery.
- For API-catalyst samples that fail to give linear dilutions and recovery, digesting the API in a chemical or microwave digestion instrument is recommended. Dilute the digested sample in 0.1 M nitric acid prior to dilution into Sample Diluent.

Dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

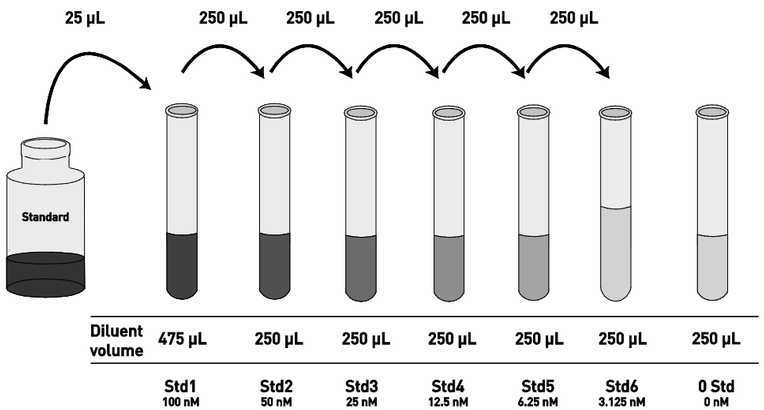
- Use all samples within **4 hours** of dilution**.**
- Samples in (or which are soluble in) water miscible solvents such as DMF, NMP, acetonitrile, DMSO, ethanol or acids (HCl, HNO_3, H_2SO_4) must be dissolved according to the following table.
- Water immiscible solvents should be treated as shown for toluene.

Solvent system	API concentration	1st Dilution required into DMF	2nd Dilution required into Sample Diluent
DMSO, DMF, NMP, MeCN	1 mg/mL	None	≥1:30
Ethanol	1 mg/mL	None	≥1:30
Acids (≤1M)	1 mg/mL	None	≥1:30
Toluene	20 mg/mL	≥1:20	≥1:30

Dilute standards

Note: Use borosilicate glass tubes for diluting standards.

1. Add 25 µL Palladium Standard to one tube containing 475 µL of Sample Diluent and label as 100 nM palladium.
2. Add 250 µL Sample Diluent to each of 6 tubes labeled as follows: 50, 25, 12.5, 6.25, 3.125, and 0 nM palladium.
3. Make serial dilutions of the standard as described below in the dilution diagram. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.



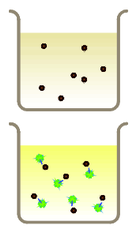
Prepare Sodium Borohydride Reagent

1. Spin vial of Sodium Borohydride Stock Solution in a microcentrifuge to ensure contents are at the bottom of the tube.
2. Add 2.5 mL of the Borohydride Buffer into a glass test tube and removing 90 µL.
3. Carefully add 90 µL of Sodium Borohydride Stock solution to the tube and vortex.
4. This volume of Sodium Borohydride Reagent is sufficient for the complete plate. Scale volumes accordingly for partial plate use.
5. Use Reagent within **2 hours** of dilution.

Assay procedure

Allow all reagents to reach room temperature before use. Mix all liquid reagents prior to use. **Total assay time is 30 minutes.**

IMPORTANT! Perform a standard curve with each assay.



Add sample

Add 100 µL of standards or diluted samples (see page 2) to the appropriate wells.

Add fluorescent detection reagent

a. Add 25 µL Palladium Detection Reagent into each well.
b. Add 25 µL Sodium Borohydride Reagent into each well.
c. Tap the side of the plate to mix. Incubate for 30 minutes at room temperature.

Target Detection Reagent

Read the plate and generate the standard curve

1. Read the fluorescent emission at 520 nm, with excitation at 485 nm.
2. Use curve-fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit. Optimally, the background fluorescence may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than that of the highest standard in the appropriate diluent and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–100 nM palladium.

Standard palladium (nM)	Mean FLU	Signal/Noise
100	19,463	192.7
50	9,564	94.7
25	4,789	47.4
12.5	2,518	24.9
6.25	1,242	12.3
3.125	608	6.0
0	101	—

Intra-assay precision

Samples of known palladium concentration were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (nM)	16.6	56.0	83.0
%CV	4.1	5.2	5.5

CV = Coefficient of Variation

Inter-assay precision

Samples were assayed 20 times in multiple assays by four operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (nM)	16.5	48.3	76.4
%CV	10.4	9.9	14.9

CV = Coefficient of Variation