

LUMINOS
INTERACTIVE ASSAY SOLUTIONS

PdX™

**Palladium API Screening
Fluorescent Detection Kit**

Catalog Number K007-F1

**Sample Types Indicated:
Screening Methods for Pd Removal in APIs**

Patent Pending: Made Under Exclusive License.

Please read this insert completely prior to using the product.

FOR RESEARCH USE ONLY

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INTRODUCTION

In recent years, many new synthetic transformations have been developed that use palladium (Pd) compounds for the catalysis of carbon-carbon and carbon-heteroatom coupling reactions such as the Buchwald-Hartwig, Heck, Kumada, Negishi, Nozaki-Hiyama, Sonogashira, Stille, Suzuki-Miyaura, and Tsuji-Trost transformations^{1,2}. These reactions have found increased popularity for pharmaceutical processes as they utilize a wide-range of functional groups and can therefore be used to build complicated molecules. However palladium-catalyzed reactions present a problem in that the palladium can often be retained in the isolated product. The LD50 values are very dependent on the physical form of the Pd compounds or catalysts used. For rats, mice, or rabbits, water soluble PdCl₂ administered intravenously gave a LD50 of 3 mg/kg body weight while relatively insoluble PdO given orally gave an LD50 >4900 mg/kg body weight³. Current European Agency for the Evaluation of Medicinal Products regulations limits all platinum group (Pt, Pd, Ir, Rh, Ru, Os) metal contamination (as a group) to less than 5 ppm⁴.

A variety of methods are available for removing Pd from active pharmaceutical ingredients (APIs). These include adsorption with trimercaptotriazine N-acetylcysteine as solids that are removed by filtration⁵, on polymeric supports⁶ such as polystyrene or as macroporous resins such as the Isolute resins (Biotage)⁷. Other adsorptive methods include activated carbon⁸, ion exchange type resins, fibrous materials such as Smopex™ (Johnson Matthey)⁹, and silica bound active resins from SiliCycle¹⁰. Typical chemical purification methods such as crystallization can also be used to lower the concentration of Pd in the final product, especially when combined with additives such as the sulfur containing ligands, N-acetylcysteine¹¹ and thiourea, and with phosphines to keep the Pd in the mother liquor.

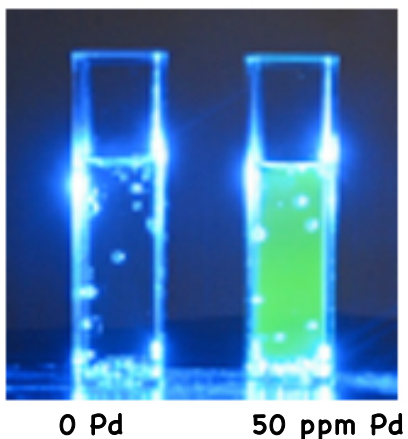
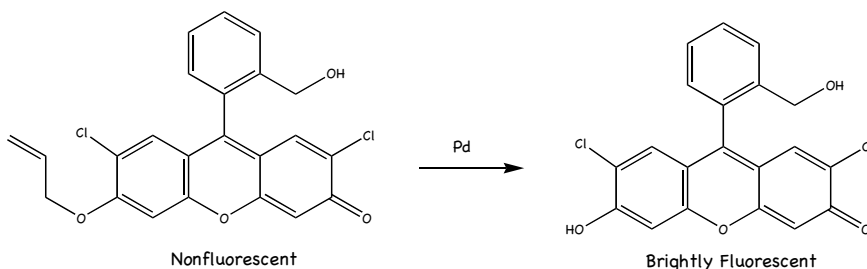
The standard methods of quantifying palladium in APIs are atomic absorption analysis, x-ray fluorescence, and plasma emission spectroscopy such as inductively-coupled plasma mass spectroscopy (ICP-MS)¹. ICP-MS is typically used both during synthesis and for final QC on drug molecules. These methods require expensive instruments with a highly trained scientist to operate. Cross-contamination of the instrument can limit the throughput of this analysis and requires scrupulous clean up methodology. In developing methods for purification protocols for APIs, the use of ICP-MS is a limit on high throughput analysis of Pd levels.

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THE LUMINOS PdX[™] PALLADIUM SCREENING FLUORESCENT DETECTION KIT

Assay Principle

The PdX[™] Palladium (Pd) Detection kit is designed to allow the rapid determination of the relative amounts of Pd present in active pharmaceutical ingredient (API) scavenging steps. Please read the complete kit insert before performing this assay. The kit uses a patent-pending, exclusively licensed non-fluorescent detection molecule that, under reducing conditions, palladium cleaves to yield a brightly fluorescent product. See the reaction below:



The relative concentrations of Pd in the sample from scavenging methods for removal of Pd is calculated, after making a suitable correction for the dilution of the sample, using software available with most plate readers.

Supplied Components

Black Microtiter Plate A 96 well plate specially coated for this assay.	96 well	Catalog Number X025-1EA
Palladium Standard Palladium chloride at 2,000 nM in 1M hydrochloric acid.	100 μ L	Catalog Number C016-100UL Caution: Caustic.
PdX™ Palladium Detection Reagent Palladium sensor solution in DMSO.	3 mL	Catalog Number C017-3ML
Sodium Borohydride Stock Solution A stock solution of sodium borohydride at 2.5M in 10M sodium hydroxide.	110 μ L	Catalog Number X026-110UL Caution: Caustic.
Borohydride Buffer Borate buffer containing stabilizers.	5 mL	Catalog Number X027-5ML
Sample Diluent A Tris buffer containing DMSO and stabilizers.	60 mL	Catalog Number X028-60ML

Storage Instructions

All components of this kit should be stored at room temperature until the expiration date of the kit.

Other Materials Required

Borosilicate glass tubes or vials.

High quality dimethyl sulfoxide, such as catalog number 41647 from the Fluka division of Sigma-Aldrich, or similar.

Fluorescence 96 well plate reader capable of reading fluorescent emission at 520 nm, with excitation at 485 nm.

Single channel, multichannel and/or repeater pipettes capable of dispensing μ L to mL quantities of liquid. Please read instructions on pages 10 and 11 for details.

Software for converting raw relative fluorescent unit (FLU) readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

Precautions

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The Palladium Standard stock is supplied in 1M hydrochloric acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

The sodium borohydride stock solution is supplied in 10M sodium hydroxide solution for stability purposes. **This solution is caustic - wear suitable laboratory clothing when handling this material.** The borohydride solution will generate hydrogen gas if diluted into acid solutions. Keep away from open flames.

Dimethyl sulfoxide is a powerful aprotic organic solvent that has been shown to enhance the rate of skin absorption of skin-permeable substances. Wear protective gloves when using the solvent especially when it contains dissolved chemicals.

Sample Types

This assay is intended to be used to evaluate scavenger methods for removing palladium catalysts from API materials. It has been validated for certain Pd catalysts dissolved in the following solvents: DMSO, DMF, NMP, Ethanol, Toluene and dilute Hydrochloric Acid. Not all chemical structures and catalyst combinations have been tried. It is up to the end user to validate the use of the kit for their combinations in the solvent conditions they are using. Refer to Page 10 for Sample Preparation directions.

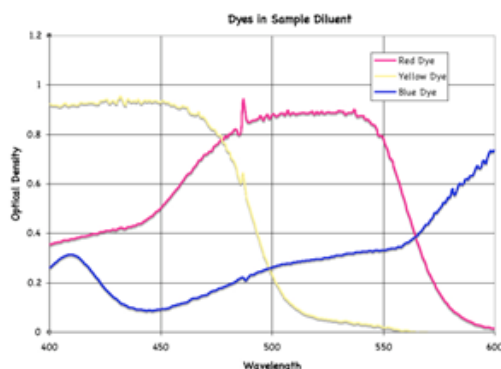
It is suggested that a reference level of the palladium content in the starting API prior to evaluation of scavenging methods be determined using ICP-MS or similar method and that this level is compared to the concentration determined using this kit. Scavenger methods can then be assessed by evaluating decreases in fluorescent signal for each method. This kit is **NOT** intended to be used as a replacement to ICP-MS for final product validation.

Interferants

To assess the effect of colored API's on the measured palladium concentration in the fluorescent assay we chose three FD&C food color preparations (Red 40 & 3 for red, Yellow 5 for yellow, and Blue 1 for blue) representing red, yellow and blue colored materials. These were added at 1:800 and 1:3200 dilutions to palladium catalyst solutions in Sample Diluent and compared to the same concentrations of palladium catalyst solutions without dyes read in the assay. The data are shown below:

Sample	Measured Pd Conc. (nM)	% Interference	Measured Pd Conc. (nM)	% Interference
	Dye diluted 1:800	Dye diluted 1:800	Dye diluted 1:3200	Dye diluted 1:3200
Sample Pd	76.2	- - -	76.2	- - -
Pd w/ Red FC	61.4	19.4	70.9	7.0
Pd w/ Yellow FC	63.4	16.8	79.7	-4.6
Pd w/ Blue FC	66.5	12.7	76.9	-0.9

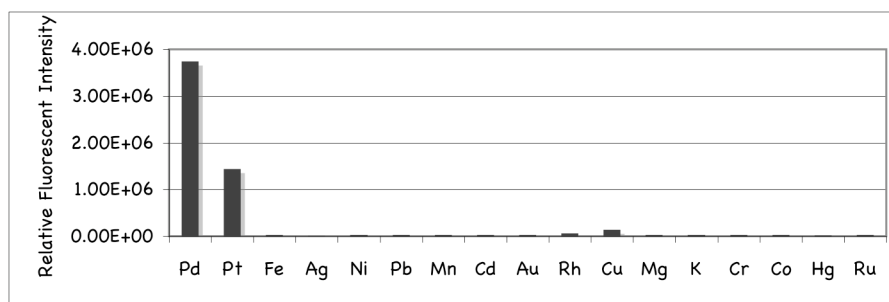
The 1:800 diluted food colorings had spectra and colors as shown below:



These dilutions are equivalent to optical densities of about 120 at 460–450 nm or about 60 at 500–540 nm in the neat solutions. These neat solutions would appear almost black to the naked eye to have any interference above 20%. We recommend that all highly colored samples be diluted in DMSO and Sample Diluent to a point where their optical densities are lower than 0.2 at 470–490 nm and/or lower than 0.1 at 510–540 nm.

Metal Specificity

The PdX™ Palladium Detection Reagent is selective for palladium and platinum over other metal ions. In the experiment below the other metal ions were run at 10 times the concentration of the palladium and platinum ions.



$\text{PdCl}_2 = 1 \mu\text{M}$; $\text{PtCl}_2 = 1 \mu\text{M}$

$\text{FeCl}_3 = \text{AgNO}_3 = \text{NiCl}_2 = \text{Pb}(\text{NO}_3)_2 = \text{MnCl}_2 = \text{CdCl}_2 = \text{AuCl}_3 = \text{RhCl}(\text{PPh}_3)_3 = \text{CuCl}_2 = \text{MgSO}_4 =$

$\text{KCl} = \text{CrCl}_3 = \text{CoCl}_2 = \text{HgCl}_2 = \text{RuCl}_3 = 10 \mu\text{M}$

Sample Preparation

The assay is designed to assess scavenging methods with samples in a variety of solvent systems: Toluene, Ethanol, DMSO, DMF, N-Methyl Pyrrolidone (NMP) or dilute hydrochloric acid. Samples in, or which are soluble in, the water miscible solvents DMSO, DMF, NMP, Ethanol or acids (HCl, HNO₃, H₂SO₄) **must** be dissolved according to the following scheme. Water immiscible solvents should be handled as shown for toluene below:

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

Solutions of API's should be made in borosilicate glass test tubes. (If using ethanol or toluene pre-fill the tip several times to ensure solvent vapor fills the space above the tip to ensure accurate delivery).

Use all samples within 2 hours of dilution.

Standard Preparation

Label six borosilicate glass test tubes as #1 through #6. Briefly spin vial of Palladium Standard in a microcentrifuge to ensure contents are at the bottom of the tube. Pipet 475 μL of Sample Diluent into tube #1 and 250 μL into tubes #2 to #6. Carefully add 25 μL of the supplied Palladium Standard solution to tube #1 and vortex completely. Take 250 μL of the 100 nM palladium standard solution in tube #1 and add it to tube #2 and vortex completely. Repeat this for tubes #3 through #6. The concentration of Pd in tubes 1 through 6 will be 100, 50, 25, 12.5, 6.25, and 3.125 nM.

Use all Standards within 2 hours of preparation.

Sodium Borohydride Reagent Preparation

Briefly spin vial of Sodium Borohydride in a microcentrifuge to ensure contents are at the bottom of the tube. Prepare the Sodium Borohydride Reagent by pipetting 2.5 mL of Borohydride Buffer into a glass test tube and removing 90 μ L. Carefully add 90 μ L of the Sodium Borohydride Stock solution to the tube and vortex.

Use Reagent within 2 hours of dilution.

Assay Protocol

We recommend that all standards and samples be run in duplicate to allow the end user to accurately assess palladium levels. Ensure that all samples have been diluted appropriately prior to running them in the assay.

1. Use the plate layout sheet on page 20 to aid in proper sample and standard identification.
2. Pipet 100 μ L of samples or standards into wells in the plate. Pipet 100 μ L of Sample Diluent into the wells for a zero standard.
3. Add 25 μ L of the PdX[™] Palladium Detection Reagent to each well, using a repeater or multichannel pipet.
4. Add 25 μ L of the Sodium Borohydride Reagent solution to each well using a repeater or a multichannel pipet.
12. Gently tap the sides of the plate to ensure adequate mixing of the reagents.
6. Incubate for 30 minutes at room temperature.
7. Read the fluorescent signal from each well in a plate reader capable of reading the fluorescent emission at 520 nm with excitation at 485 nm. Please contact your plate reader manufacturer for suitable filter sets.
8. Use the plate reader's built-in 4PLC software capabilities to calculate Pd concentration for each sample.

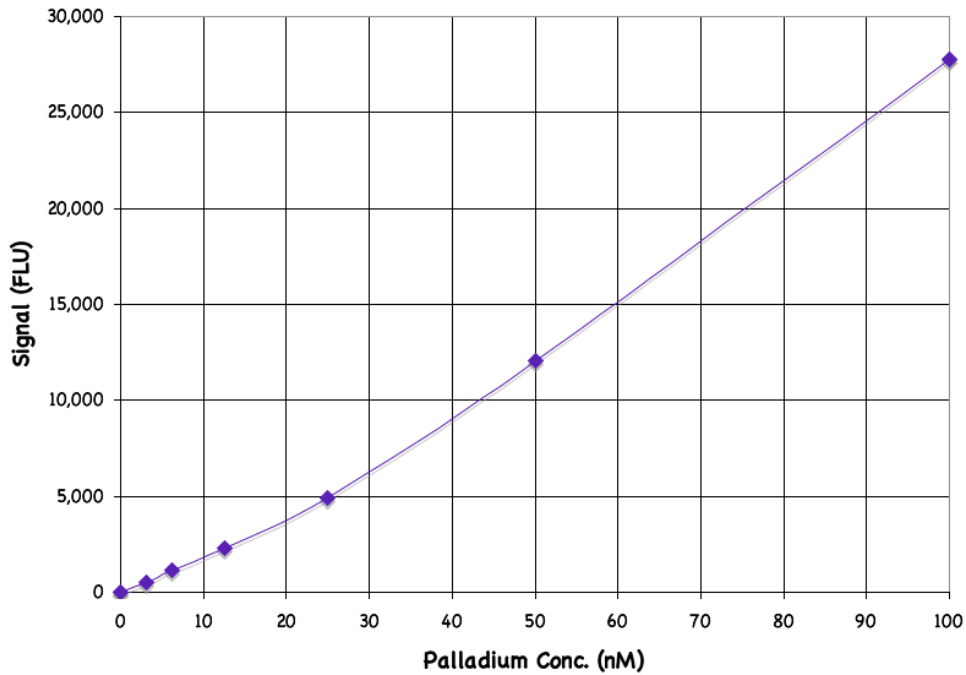
Calculation of Results

Average the duplicate FLU readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader. The sample concentrations obtained should be multiplied by the dilution factor to obtain sample values.

Sample	Mean FLU	Net FLU	Pd Concentration (nM)
Zero Std	241	-	0
Std 1	28,009	27,769	100
Std 2	12,299	12,058	50
Std 3	5,147	4,907	25
Std 4	2,559	2,319	12.5
Std 5	1,370	1,129	6.25
Std 6	746	506	3.125
Sample 1	19,257	19,016	72.6
Sample 2	3,183	2,943	16.1

**Always run your own standard curve for calculation of results.
Do not use this data.**

Typical Standard Curve



Always run your own standard curve for calculation of results. Do not use this data.

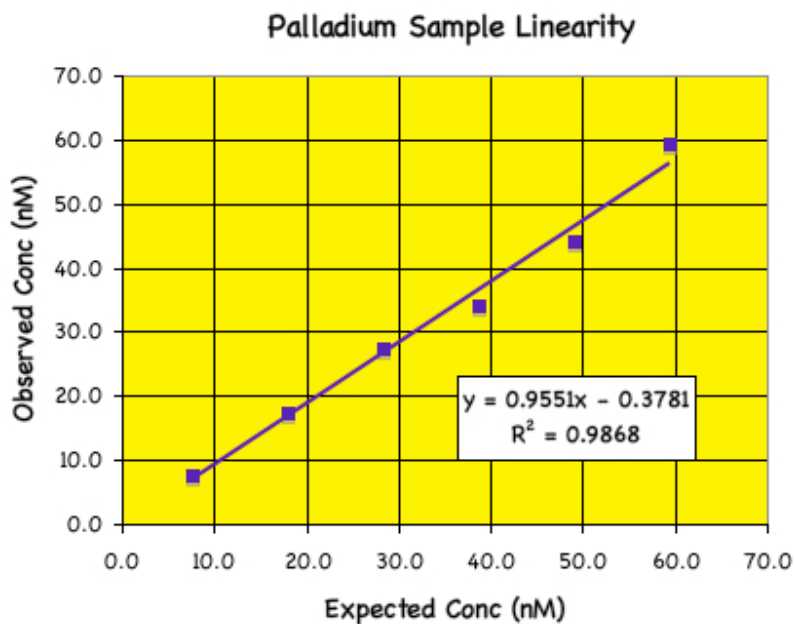
Example

A sample of an API diluted according the directions on Page 10 at 1 mg/mL in DMSO with 100 ppm Pd contamination would have a Pd concentration of 100 ng/mL which is equivalent to 939.7 nM. After being diluted 1:30 in Sample Diluent this sample would read at 31.3 nM.

Linearity

Linearity was determined by taking two palladium catalyst samples in toluene, diluted 1:20 in DMSO, and then diluted in Sample Diluent, one, Bis(dibenzylideneacetone) palladium(0), with a low diluted Pd level of 7.6 nM and one, 2-(2'-Di-tert-butylphosphine)biphenylpalladium(II) acetate, with a higher diluted level of 59.5 nM and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

Low Pd Sample	High Pd Sample	Expected Conc. (nM)	Observed Conc. (nM)	% Recovery
100%	0%	7.6	---	---
80%	20%	18.0	17.2	95.7
60%	40%	28.4	27.5	97.0
40%	60%	38.7	34.0	87.8
20%	80%	49.1	44.2	90.0
0%	100%	59.5	---	---
			Mean Recovery	92.6



Intra Assay Precision

Three different palladium catalyst samples were diluted with Sample Diluent and run in replicates of n=20 in an assay. The mean and standard deviation of the calculated Pd concentrations were:

Sample	Palladium Conc. (nM)	Standard Deviation	%CV
1	16.6	0.7	4.1
2	56.0	2.9	5.2
3	83.0	4.6	5.5

Inter Assay Precision

Three different palladium catalyst samples were diluted with Sample Diluent and run in duplicates in 20 assays run over two days by four operators. The mean and standard deviation of the calculated Pd concentrations were:

Sample	Palladium Conc. (nM)	Standard Deviation	%CV
1	16.5	1.7	10.4
2	48.3	4.8	9.9
3	76.4	11.4	14.9

Catalyst Recovery

Five Pd catalysts were dissolved in DMSO to a catalyst concentration of 1mg/mL and the concentration of Pd was calculated. Each catalyst was diluted to working concentrations of 20 and 5 nM Pd in Sample Diluent and run in the assay alongside PdCl₂ control dilutions of the same working concentrations. The obtained values were evaluated for linearity off the lowest concentration (5 nM) and for percent recovery as compared to the control values.

	Nominal Pd Conc (nM)	Observed Conc (nM)	Linearity	Obs.Cntrl Conc (nM)	%Recovery
Catalyst 1	20.00	16.81	94.1%	19.54	86.0
Catalyst 1	5.00	4.47	---	5.18	86.2
Catalyst 2	20.00	16.17	99.8%	---	80.9
Catalyst 2	5.00	4.05	---	---	81.0
Catalyst 3	20.00	16.67	107.4%	18.56	89.8
Catalyst 3	5.00	3.88	---	5.18	74.9
Catalyst 4	20.00	13.80	110.8%	19.97	69.1
Catalyst 4	5.00	3.12	---	4.97	62.7
Catalyst 5	20.00	17.87	91.8%	19.39	92.2
Catalyst 5	5.00	4.87	---	4.80	101.3

Palladium catalysts were:

#1 = Allylpalladium(II) chloride dimer

#2 = Bis-(dibenzylideneacetone)palladium(0)

#3 = Bis(triphenylphosphine)palladium(II) dichloride

#4 = 2-(2'-Di-tert-butylphosphine) biphenylpalladium(II) acetate

#5 = Palladium(II) acetate

API Interaction Tests

We obtained 5 chemical compounds that bind extremely tightly to palladium catalysts and could be representative of structures found in APIs. These 5 chemical entities were A, 2-methyl-2-oxazoline; B, thiazole; C, 4,4'-diphenyl-2,2'-dipyridyl; D, 8-aminoquinoline; and E, thiazolidine-2-carboxylic acid. The structures are shown on page 18.

Each of the 5 compounds (A-E) were dissolved in DMSO. Each compound was then spiked with each of 5 catalysts (from pg 16) and the palladium standard, PdCl₂ to concentrations equivalent to 10 ppm Pd contamination. Each compound without added palladium catalysts was run as compound controls. Each catalyst spiked into DMSO without a compound was run as catalyst controls.

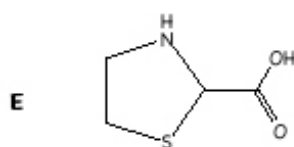
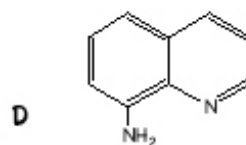
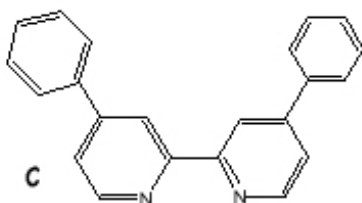
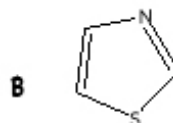
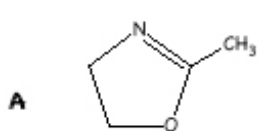
Multiple dilutions of each of the solutions were made into Sample Diluent and run in the assay. The obtained values for each compound + catalyst, as well each compound control, were evaluated for recovery against the catalyst control values. No signal was detected for compound only controls.

Average Recovery off Controls					
Catalyst* or Standard	Compound A 50 mg/mL	Compound B 50 mg/mL	Compound C 5 mg/mL	Compound D 50 mg/mL	Compound E 5 mg/mL
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

* = See page 16 for palladium catalyst details

ND = Not detectable.

Compound Structures



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

LIMITED WARRANTY

Luminos LLC warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.



CONTACT INFORMATION

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PLATE LAYOUT

H	G	F	E	D	C	B	A	
								1
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