# HPLC Application

# TMP and Thiamine Standard 5 nmol/L by Impact PPT on Kinetex 5u C18 100x4.6mm

Column: Dimensions: Order No: Elution Type: Eluent A: Eluent B:	100 x 4.6 m 00D-4601-E Gradient 25mM Na2H		ol, pH 7.0	4.6 mm, Ea		Kinetex* Ultra-High Performance on Any LC System
Gradient	Step No.	Time (min)	Pct A	Pct B		
Profile:	1	0	97	3		
	2	0.25	75	25		Products used in this application:
	3	0.75	75	25		Products used in this application.
	4	3	65	35		
	5	4	40	60		KINETEX.
	6	4.5	97	3		
	7	5	97	3		
Flow Rate: Col. Temp.: Detection: Analyst Note: 20881 56.0 55.0 55.0		e (FLUOR) @ Ex. 3 rotein Precipitation Plate (	•	•	ration2	
0		1	2	3	4	5 min

### **ANALYTES:**

1 Thiamine monophosphate Retention Time: 2.658 min

# 2 Thiamine

Retention Time: 4.312 min

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for HPLC Application ID No.: 20881



# TMP and Thiamine Standard 5 nmol/L by Impact PPT on Kinetex 5u C18 100x4.6mm

### **PRODUCT DESCRIPTION:**

Impact<sup>™</sup> Protein Precipitation, 2mL Square Well Filter Plate, 2/Pk

Order No.: CE0-7565

## SOLID PHASE EXTRACTION (SPE) PRODCEDURE:

Note: The solvent volumes shown below are for a Proprietary bed mass.

The solvent volumes will need to be adjusted for a smaller or larger bed mass.

#### Condition:

Load:	
Wash:	
Dry:	
Elute:	

#### Final Prep and Analysis:

Add  $400\mu$ L of methanol to a well of a Protein Precipitation Plate; Add  $100\mu$ L plasma to the well. Mix 3 times with pipette tip (or vortex the whole plate briefly); Wait for 5 minutes; Filter through Inject: 20 µL on HPLC Fluorescence (FLUOR) @ Ex. 375 nm (ambient)

ANALYTES:	Spiked Conc. (ng/mL)	Log P	рКа	% Rec	%RSC (n=0)
1 Thiamine monophosphate	5				
2 Thiamine	5				

**Note:** This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals. Call your local Phenomenex Representative for assistance in method development and optimization techniques.

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