



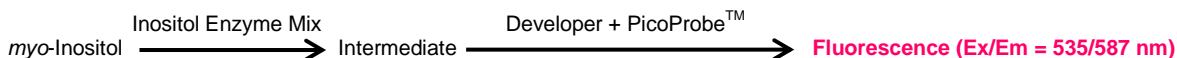
PicoProbe™ *myo*-Inositol Assay Kit (Fluorometric)

10/18

(Catalog # K469-100; 100 assays; Store at -20°C)

I. Introduction:

Inositol is a carbocyclic sugar that is also known as vitamin B8 and is believed to have numerous health benefits. This substance must be obtained either from exogenous sources, or generated from metabolism of glucose-6-phosphate. While several isomers exist, *myo*-Inositol is the only biologically relevant form. When metabolized, it becomes a central component of multiple secondary signaling molecules, including inositol phosphates and phosphatidylinositol. These compounds participate in a number of eukaryotic signaling pathways, from insulin signaling and gene expression to cytoskeletal assembly and intracellular calcium mediation. BioVision's PicoProbe *myo*-Inositol Assay Kit gives the user a specific, rapid, high-throughput adaptable method for determination of this molecule in biological samples. A Sample Clean-Up Mix is provided for easy removal of glucose in the sample that may contribute to background. Our assay will quantify amounts of inositol as low as 20 pmoles.



II. Applications:

- Measurement of *myo*-Inositol content in liquid samples and from cell and tissue lysates

III. Sample Type:

- Biological Fluids (Breast Milk, Saliva, Plasma or Serum)
- Tissue or Cell Lysate

IV. Kit Contents:

Components	K469-100	Cap Code	Part Number
Inositol Assay Buffer	25 ml	WM	K469-100-1
Inositol Enzyme Mix	1 vial	Green	K469-100-2
Inositol Developer	1 vial	Red	K469-100-3
PicoProbe™ (in DMSO)	400 µl	Blue	K469-100-4
Sample Clean-Up Mix	1 vial	Orange	K469-100-5
Inositol Standard	1 vial	Yellow	K469-100-6

V. User Supplied Reagents and Equipment:

- 96-well flat bottom white plate
- Multi-well spectrophotometer
- PBS (BV #2113)
- Dounce Tissue Homogenizer (BV #1998)
- 10 kD Spin Column (BV #1997)

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. Centrifuge vials prior to opening. Read the protocol before performing the assay.

- **Inositol Assay Buffer:** Warm to room temperature before use.
- **Inositol Enzyme Mix, Inositol Developer and Sample Clean-Up Mix:** Store at -20°C. Lyophilized vials are stable for at least 6 months. Reconstitute Inositol Enzyme Mix, Inositol Developer, and Sample Clean-Up Mix in 220 µl assay buffer each before use. Pipet up and down and vortex to fully reconstitute. Reconstituted vials are stable for at least two months at -20°C.
- **PicoProbe™ (in DMSO):** Store at -20°C. Thaw at room temperature before use. Aliquot and store at -20°C.
- **Inositol Standard:** Reconstitute with 200 µl dH₂O to generate 100 mM (100 nmol/µl) Inositol Standard solution. Keep on ice while in use. Store at -20°C. Stable for at least two months.

VII. *myo*-Inositol Assay Protocol:

1. **Sample Preparation:** Liquid samples can be assayed directly. **For tissue or cell samples:** 10 mg tissue or 1x10⁶ cells should be rapidly homogenized with 100 µl ice-cold Assay Buffer. Centrifuge at 10 000 x g at 4°C for 5 min to remove insoluble materials. Most samples will require treatment with Sample Clean-Up Mix; add 2 µl Sample Clean-Up Mix to 100 µl of clarified sample and incubate for 1 hour at 37°C. Filter through 10 kD MWCO spin column (Cat. # 1997) at 10000 x g for 10 min. at 4°C. Add 2 - 20 µl filtrate into duplicate wells of a 96-well white plate, bring volume to 50 µl with Assay Buffer. *For unknown samples, we suggest testing several doses of your samples to ensure readings are within the standard curve range.*
2. ***myo*-Inositol Standard Curve Generation:** Dilute the 100 mM Inositol standard by adding 10 µl to 990 µl Assay Buffer to obtain 1 mM Inositol Standard. Further dilute the 1 mM Inositol Standard 1:20 by adding 10 µl to 190 µl Assay Buffer (50 µM standard). Add 0, 2, 4, 6, 8, and 10 µl of the 50 µM Inositol standard to wells of the 96 well plate to obtain 0, 100, 200, 300, 400, and 500 pmol of *myo*-inositol per well. Bring up the total volume in these wells to 50 µl with Inositol Assay buffer.

3. Reaction mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 μl . The total reaction volume after addition of reaction mix is 100 μl .

	Reaction Mix	Background Mix
Inositol Assay Buffer	43 μl	45 μl
Inositol Enzyme mix	2 μl	--
Inositol Developer	2 μl	2 μl
PicoProbe™ (in DMSO)	3 μl	3 μl

Add 50 μl of the reaction mix to standard and sample total signal wells. Add 50 μl Background Mix to background (second) sample wells. Mix well. Incubate at 37°C for 30 minutes.

4. Measurement: Record fluorescence in end point mode at Ex/Em= 535/587 nm.

5. Calculations: Subtract 0 Inositol reading from all Inositol standard readings. Plot the *myo*-Inositol Standard Curve. Subtract sample background control readings from sample readings. Apply corrected RFU to Standard Curve to get B nmol Inositol in the sample well.

$$\text{Inositol concentration in sample (C)} = (\text{B} / \text{V}) \times \text{D} \text{ (pmol/}\mu\text{l) or } \mu\text{M}$$

Where **B** = Amount of *myo*-Inositol in the sample well from Standard Curve (nmol)

V = Volume of sample added into the well (μl)

D = Dilution factor

Inositol concentrations can also be expressed as pmol Inositol per μg protein (nmole per mg)

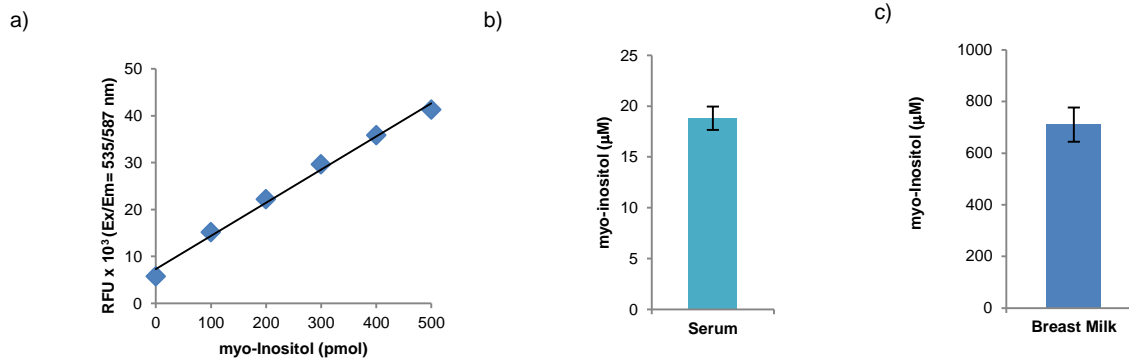


Figure: (a) *myo*-Inositol standard curve. (b) *myo*-Inositol content in pooled human serum. Serum was treated with Sample Clean-Up Mix and passed through a 10 kDa spin column and diluted 10-fold. 5-20 μl of the filtrate was run in the assay. (c) *myo*-Inositol determination in breast milk. 20 μl human breast milk was diluted 5-fold with PBS and treated with Sample Clean-Up Mix according to the above protocol, and after centrifugation, 2-8 μl of the filtrate was assayed.

VIII. RELATED PRODUCTS

Glucose Colorimetric/Fluorometric Assay Kit (K606)
Glucose Colorimetric Assay Kit II (K686)
PicoProbe™ Glucose Fluorometric Assay Kit (K688)
 β -xylosidase Activity Assay Kit (K981)
Fructose Colorimetric/Fluorometric Assay Kit (K619)

β -Glucuronidase Activity Assay Kit (K514)
Fructose Assay Kit (Colorimetric) (K439)
PicoProbe™ Fructose Fluorometric Assay Kit (K611)
PicoProbe™ Fructose-6-Phosphate Fluorometric Assay Kit (K689)
PicoProbe™ Glucose-6-phosphate Fluorometric Assay Kit (K687)

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