Store at 4°C

PTMScan® Phospho-Enrichment IMAC Fe-NTA Magnetic Beads

#20432

Small 200 µl (10 assays)
Large 200 µl x 5 (50 assays)

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

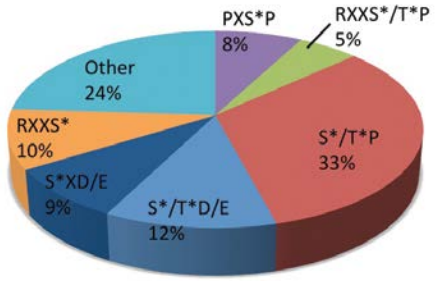
Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

rev. 12/04/17

For Research Use Only. Not For Use In Diagnostic Procedures.

Description: PTMScan® Phospho-Enrichment IMAC Fe-NTA Magnetic Beads employ immobilized metal affinity chromatography for capturing phosphorylated peptides. Negatively charged phosphate groups are attracted to the positively charged metal ions on the beads. In conjunction with liquid chromatography tandem mass spectrometry (LC-MS/MS), this approach enables researchers to isolate, identify, and quantitate large numbers of phosphorylated cellular peptides with a high degree of specificity and sensitivity, providing a global overview of phosphorylation in cell and tissue samples. For more information on PTMScan® Proteomics Services, please visit https://www.cellsignal.com/services/index.html.

Background: Immobilized metal affinity chromatography, or IMAC, has been widely used to enrich proteins and peptides from biological samples by binding to clusters of negative charge. Divalent transition metal ions Co2+, Cu2+, Ni2+, and Zn2+ are often used to purify proteins rich in poly-Histidine or Cysteine as well as proteins with metal affinity. Trivalent metal ions, Fe3+, Ga3+, Al3+, as well as Ti4+ and Zr4+ are commonly used for phospho-peptide enrichment for proteomic studies (1). Iminodiacetic acid (IDA) or nitrilotriacetic acid (NTA) are used for chelating the metal ions to agarose-coated beads. In comparison studies, NTA has been shown to perform better than IDA at selectively capturing and identifying more phospho-peptides. Ga3+ and Fe3+ are comparable with respect to the number of phospho-peptides identified (2). Compared to metal oxide affinity chromatography (MOAC) using TiO2, Fe3+ IMAC performed marginally better with TiO2 having a preference for acidic phospho-peptides (pI > 4) relative to Fe3+ which prefered less acidic peptides (pI < 4) (3). The PTMScan® Fe-NTA Magnetic Beads offer an efficient tool for phospho-peptide enrichment with little or no bias for phospho-residue context. They can be employed independently or in conjunction with immunoaffinity based enrichment to complement any PhosphoScan® LC-MS/MS proteomic study.



This chart shows the underlying motif distribution in a PhosphoScan® IMAC LC-MS/MS experiment using 4026 nonredundant tryptic peptides generated from mouse embryo and captured using PTMScan® Phospho-Enrichment IMAC Fe-NTA Magnetic Beads. Within this data set, 84% of the peptides were phosphorylated on serine residues, 13% on threonine, and 3% on tyrosine.

The Motif Logo was generated from a PhosphoScan® IMAC LC-MS/MS experiment using 4026 nonredundant tryptic peptides derived from mouse embryo and captured using PTMScan® Phospho-Enrichment IMAC Fe-NTA Magnetic Beads. The logo demonstrates the relative frequency of amino acids in a given position around the modified site within this data set.

Storage: IMAC Fe-NTA Magnetic Beads are supplied in 20% ethanol. For long term storage, replace the buffer with long term storage buffer (1:1:1 v/v/v of methanol, acetonitrile, and 0.01% acetic acid). See protocol for details. Store at 4°C. Do not freeze the beads.

Directions for use: Cells are lysed in a urea-containing buffer, cellular proteins are digested by proteases, and the resulting peptides are purified by reversed-phase solid-phase extraction. Phospho-peptides are then subjected to enrichment using PTMScan® IMAC Beads. Unbound peptides are removed through washing, and the captured phospho-peptides are eluted with basic pH buffer. Reversed-phase purification is performed on microtips to desalt and separate peptides from impurities prior to concentrating the enriched peptides for LC-MS/MS analysis. A detailed protocol is included with the beads.

Background References:

(1) Block, H. et al. (2009) *Methods Enzymol* 463, 439-73.

(2) Ficarro, S.B. et al. (2009) *Anal Chem* 81, 4566-75.

(3) Yue, X. et al. (2015) *Anal Chem* 87, 8837-44.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

NOTE: For long-term storage, it is recommended to replace the IMAC Fe-NTA Magnetic Bead storage buffer with an equal ratio mix (1:1:1 v/v/v) of acetonitrile, methanol, and 0.01% acetic acid. Prepare solutions and perform washes with HPLC grade reagents. Remove the storage buffer and wash the beads with 1 mL water three times. Remove the water wash and add 0.2 mL long-term storage buffer to each 10 assay tube. Store at 4°C.

A. Solutions and Reagents

Reagents Not Included:

1. HEPES (Sigma, H-4034)
2. Sodium pyrophosphate (Sigma, S-6422)
3. β-glycerophosphate (Sigma, G-9891)
4. Urea, Sequanal grade (Thermo Scientific, 29700)
5. Sodium orthovanadate (Sigma, S-6508)
6. Iodoacetamide (Sigma, I-6125)
7. Dithiothreitol (DTT) (Cell Signaling Technology, 7016)
8. Trypsin-TPCK (Worthington, LS-003744)
9. Trypsin (Sequencing Grade, Promega V511C, V5113)
10. Trifluoroacetic acid (TFA), Sequanal grade (Thermo Scientific, 28903)
11. Trifluoroacetic Acid (TFA), Reagent grade (American Bioanalytical, AB02010)
12. Acetonitrile (Thermo Scientific, 51101)
13. Sep-Pak(R) C18 Vac cartridge, 100 mg (Waters, WAT023590)
14. Burdick and Jackson Water (Honeywell, AH365-4)
15. 1 mM Hydrochloric acid (HCL)
16. Ammonium bicarbonate (Sigma, A-6141)
17. Ammonium hydroxide 28% (Sigma, 338818)
18. Acetic Acid, (J.T. Baker, 6903-05)
19. Burdick and Jackson Methanol (Honeywell, 230)
20. Magnetic tube rack (CST #7017, #14654)

NOTE: Prepare solutions for cell lysis (Section I) and Sep-Pak purification (Section II) with reverse osmosis deionized (RODI) or equivalent grade water. Prepare solutions using HPLC grade water (Burdick and Jackson) for the IMAC bead enrichment (Section III) and the peptide concentration steps (Section IV).

Stock Solutions:

1. **HEPES, pH 8.0: Make 10X stock (200mM):** Dissolve 23.8 g HEPES in approximately 450 ml water, adjust to pH 8.0 with 5 M NaOH, and bring to a final volume of 500 ml. Filter through a 0.22 µM filter. Store at 4°C for use up to six months.
2. **Sodium pyrophosphate: Make 50X stock (125 mM):** 1.1 g/20 ml. Store at 4°C for up to six months.
3. **β-glycerophosphate:** Make 1000X stock: 2.2 g/10 ml. Divide into 100 µl aliquots and store at -20°C.
4. **Sodium orthovanadate:** Make 100X stock: 1.84 g/100 ml. Sodium orthovanadate must be depolymerized (activated) according to the following protocol:
   a. For a 100 ml solution, fill up with water to approximately 90 ml. Adjust the pH to 10.0 using 1 M NaOH with stirring. At this pH, the solution will be yellow.
   b. Boil the solution until it turns colorless and cool to room temperature (put on ice for cooling).
   c. Readjust the pH to 10.0 and repeat step 2 until the solution remains colorless and the pH stabilizes at 10.0 (usually it takes two rounds). Adjust the final volume to 100 ml with water.
   d. Store the activated sodium orthovanadate in 1 ml aliquots at -20°C for up to six months. Thaw one aliquot for each experiment; do not refreeze thawed vial.
5. **Dithiothreitol (DTT):** Make 1.25 M stock: 19.25 g/100 ml. Divide into 200 µl aliquots. Store at -20°C for up to one year. Thaw one aliquot for each experiment.
   Trypsin-TPCK (Worthington): Store dry powder for up to 2 years at -80C. Seal the cap of the trypsin-TPCK container with parafilm to avoid collecting moisture, which can lead to degradation of the reagent. Prepare 1 mg/mL stock in 1 mM HCl. Divide into 1 mL aliquots. Store at -80C for up to 1 year.
6. **Trypsin-TPCK (Worthington):** Store dry powder for up to 2 years at -80C. Seal the cap of the trypsin-TPCK container with parafilm to avoid collecting moisture, which can lead to degradation of the reagent. Prepare 1 mg/mL stock in 1 mM HCl. Divide into 1 mL aliquots. Store at -80C for up to 1 year.

I. Cell Lysis and Protein Digestion

A. Solutions and Reagents

NOTE: Prepare solutions with RODI or equivalent grade water.

1. **Urea Lysis Buffer:** 20 mM HEPES pH 8.0, 9 M urea, 1 mM sodium orthovanadate, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate.

   NOTE: The Urea Lysis Buffer should be prepared fresh prior to each experiment. Do not include protease inhibitors.

   NOTE: Dissolving urea is an endothermic reaction. Urea Lysis Buffer preparation can be facilitated by placing a stir bar in the beaker and by using a warm (not hot) water bath on a stir plate. 9 M urea is used so that upon lysis, the final concentration is approximately 8 M. The urea lysis buffer should be used at room temperature. Placing the urea lysis buffer on ice will cause the urea to precipitate out of solution.
2. **DTT solution (1.25 M)** (see stock solutions for preparation)
3. **Iodoacetamide solution:** Dissolve 95 mg of iodoacetamide in water to a final volume of 5 ml. After weighing the powder, store in the dark and add water only immediately before use. The iodoacetamide solution should be prepared fresh prior to each experiment.

B. Cell Harvesting

Note: DO NOT place Urea Lysis Buffer or culture dishes on ice during harvesting. Harvest cells using Urea Lysis Buffer at room temperature. During lysis, the buffer becomes viscous due to DNA released from the cells.

Suspension Cells:

1. Grow 1-2 X 10^7 cells for each experimental condition (enough cells to produce 1-2 mg of soluble protein). Cells grown to 70-80% confluence in a large 150 mm dish can be used to obtain enough or excess material (depending on the cell type).
2. Harvest culture dishes for each sample, remove media from each dish by aspirating or decanting, and tilt the dish so the remaining medium flows to the bottom edge. Aspirate/remove the remainder of the medium. Rinse each dish with cold PBS. Remove all the remaining PBS as described above.
3. Add 1 mL of Urea Lysis Buffer (at room temperature) to one 15 cm dish, scrape the cells into the buffer, and tilt the dish to scrape and collect all the lysate. Using a trimmed P1000 tip, transfer the lysate to a 1.7 mL microcentrifuge tube.

C. Sonication of Cell Lysate

NOTE: Lysate sonication fragments DNA and reduces sample viscosity. Ensure that the sonicator tip is submerged in the lysate. If the sonicator tip is not submerged properly, it may induce foaming and reduce the effectiveness of the sonication. In order to prevent splashing during sonication, if the volume of lysate exceeds 1.3 mL in a 1.7 mL microcentrifuge tube, split sample into two tubes and recombine post-sonication.

1. Using a microtip, sonicate lysate at 15 W output with 3 bursts of 15 sec each.
2. Clear the lysate by centrifugation in a microcentrifuge at 16,000 – 20,000 x g for 15 min at room temperature, then transfer the supernatant (protein extract) into a new 15 mL conical tube.

   NOTE: If desired, the PTMScan® protocol may be interrupted at this stage. The cell lysate can be frozen and stored at -80°C for several weeks. A protein assay is recommended to determine protein concentration for each sample to ensure the amount of material available is adequate for the following steps

D. Reduction and Alkylation of Proteins

1. Add 1/278 volume of 1.25 M DTT to the cleared cell supernatant (e.g. 36 µl of 1.25 M DTT for 10 ml of protein extract), mix well and place the tube into a 55ºC incubator for 30 min.
2. Cool the solution on ice briefly until it has reached room temperature.
3. Add 1/10 volume of iodoacetamide solution to the cleared cell supernatant, mix well, and incubate for 15 min at room temperature in the dark.

E. Trypsin Digestion

1. Dilute 4-fold with 20 mM HEPES pH 8.0 to a final concentration of approximately 2 M urea, 20 mM HEPES, pH 8.0. For example, add 30 ml 20 mM HEPES pH 8.0 for 10 ml of lysate.
2. Add 1/100 volume of 1 mg/mL Trypsin-TPCK (Worthington, LS003744) stock in 1 mM HCl and digest overnight at room temperature with mixing.
3. Analyze the lysate before and after digest by SDS-PAGE to check for complete digestion.

II. Sep-Pak® C_{18} Purification of Lysate Peptides

A. Solutions and Reagents

NOTE: Prepare solutions with RODI or equivalent grade water. Use Trifluoroacetic acid (TFA), Reagent grade (American Bioanalytical, AB02010) and PierceTM Acetonitrile (ACN), LC-MS Grade (Thermo Scientific, 51101) when preparing solutions. All percentage specifications for solutions are vol/vol. Organic solvents are volatile. Tubes containing small volumes of these solutions should be prepared immediately before use and should be kept capped as much as possible because the organic components evaporate quickly.

1. **20% trifluoroacetic acid (TFA):** add 10 ml TFA to water to a total volume of 50 ml.
2. **Solvent A (0.1% TFA):** add 1 mL of 20% TFA to 199 mL water.
3. **Solvent B (0.1% TFA, 40% acetonitrile):** add 40 mL of acetonitrile (ACN) and 0.5 mL of 20% TFA to 50 mL of water, adjust final volume to 100 mL with water.
4. **Wash buffer (0.1% TFA, 5% acetonitrile):** For 100 ml of wash buffer, add 0.5 ml of 20% TFA to 50 ml of water, then add 5 ml of acetonitrile, adjust final volume to 100 ml with water.

B. Acidification of Digested Cell Lysate

NOTE: Before loading the peptides from the digested sample on the column, they must be acidified with TFA for efficient peptide binding. The acidification step helps remove fatty acids from the digested peptide mixture.

1. Add 1/20 volume of 20% TFA to the digest for a final concentration of 1% TFA. Check the pH by spotting a small amount of peptide sample on a pH strip (the pH should be under 3). After acidification, allow precipitate to form by letting sample stand on ice for 15 min.
2. Centrifuge the acidified peptide solution for 15 min at 1,780 x g at room temperature to remove the precipitate. Transfer peptide-containing supernatant into a new 15 mL conical tube without dislodging the precipitated material.

C. Peptide Purification

Purification of peptides is performed at room temperature on Sep-Pak C18 cartridges or columns from Waters Corporation. For samples of 1-2 mg, a 100 mg sorbent cartridge can be used (WAT023590). Purification on C18 columns should be performed using gravity flow. In cases when flow rate slows, the C18 cartridge can be connected to a vacuum pump. For faster flow rates during loading and washing of the Sep-Pak column, load up to 1 mg of the digested peptides. For larger sample sizes, higher capacity columns may be used (eg. 360 mg SEP-PAK Classic C18, WAT051910) and the volume of wash and elution buffers should be adjusted accordingly. Sep-Pak® C18 purification uses reversed-phase (hydrophobic) solid-phase extraction. Peptides and lipids bind to the chromatographic material. Large molecules such as DNA, RNA, and most protein, as well as hydrophilic molecules such as many small metabolites are separated from peptides using this technique. Peptides are eluted from the column with 40% acetonitrile (ACN) and separated from lipids and proteins, which elute at approximately 60% ACN and above. Purify peptides immediately after proteolytic digestion.

1. Pre-wet the column with 1 mL 100% ACN.
2. Wash sequentially with 1 mL Solvent A (0.1% TFA) 3 times.

3. Load acidified and cleared digest (from Section B).
4. Wash sequentially with 1 mL, of Solvent A (0.1% TFA) 2 times.
5. Wash sequentially with 1 mL of wash buffer (0.1% TFA, 5% ACN) once.
6. Place columns above new 15 mL polypropylene tubes to collect the eluate. Elute peptides sequentially with 0.5 mL of Solvent B (0.1% TFA, 40% ACN) 2 times.
7. Freeze the eluate on dry ice (or -80°C freezer) for 2 hr to overnight and lyophilize frozen peptide solution for a minimum of 2 days to assure TFA has been removed from the peptide sample.

NOTE: The lyophilization should be performed in a standard lyophilization apparatus. **DO NOT USE a SPEED-VAC apparatus at this stage of the protocol**.

NOTE: Lyophilized, digested peptides are stable at -80°C for several months (seal the closed tube with parafilm for storage). The PTMScan® procedure can be interrupted before or after lyophilization.

III. IMAC Fe-NTA Magnetic Bead Purification:

A. Solutions and Reagents

NOTE: Prepare solutions with HPLC grade water. Trifluoroacetic acid and acetonitrile should be of the highest grade (Sequanal). All percentage specifications for solutions are vol/vol.

1. **Loading Buffer: (0.1% TFA, 85% acetonitrile)** For 5 mL, combine 4.25 mL ACN, 0.725 mL water, and 25 µL 20% TFA.
2. **Wash Buffer: (0.1% TFA, 80% acetonitrile)** For 50 mL, combine 40 mL ACN, 9.75 mL water, and 250 µL 20% TFA.
3. **Elution Buffer: (50% acetonitrile, 2.5% ammonia)** For 5 mL, combine 0.45 mL 28% ammonia (Sigma, 338818) with 2.05 mL water then add 2.5 mL ACN.
4. Prepare fresh 1 M ammonium bicarbonate (AMBIC) stock solution.
5. Prepare 5% TFA by adding 50 µL 20% TFA to 150 µL water

B. Procedure (for secondary trypsin digest)

NOTE: Prior to IMAC enrichment, a secondary digest with sequencing grade trypsin is necessary to minimize missed cleavage sites.

1. Prepare sequencing grade trypsin digestion solution (5% ACN, 50 mM ammonium bicarbonate, and 100 ng/uL trypsin). Combine 33 µL water with 2.5 µL 1M ammonium bicarbonate, 2.5 µL ACN and 12 µL (5 µg) Trypsin (Promega, V511C).
2. Centrifuge 0.5 mg lyophilized peptide in a 1.7 mL microcentrifuge tube for 5 minutes at 2,000 x g at room temperature to pellet all material at the bottom of the tube. Resuspend pellet in 50 µL trypsin digestion solution mechanically by pipetting a few times with a micropipettor.

NOTE: After dissolving the peptide, check the pH of the peptide solution by spotting a small volume (1-2 µL) on pH indicator paper, the pH should be close to 8.0 or no lower than 6.0. In the rare case that the pH is more acidic (due to insufficient removal of TFA from the peptide under sub-optimal conditions of lyophilization), titrate the peptide solution with 1 M AMBIC solution that has not been adjusted for pH. 1-2 µL is usually sufficient to neutralize the solution.

3. Perform the digest at 37° C for two hours. Terminate the reaction and acidify the solution by adding 5 µL 5% TFA.
4. Add 0.95 mL Loading Buffer (0.1% TFA, 85% ACN) to the digested peptide solution and vortex.
5. Clear solution by centrifugation for 5 min at 10,000 x g at 4°C in a microcentrifuge. The insoluble pellet may appear considerable. This will not pose a problem since most of the peptide will be soluble.
6. Resuspend PTMScan® IMAC Fe-NTA Magnetic Beads by rotating end over end on a rotator 10 minutes. Using a trimmed P20 pipette tip, take 20 µL of 25% bead slurry (5 µL packed beads) and transfer to a 1.7 mL microcentrifuge tube.
7. Wash the IMAC magnetic beads with 1 mL Wash Buffer 3 times. Allow the beads to settle on the magnetic stand (CST #7017, #14654). Remove the washes taking care not to remove any beads.
8. Taking care not to dislodge any pelleted material, transfer the cleared peptide solution into the vial containing IMAC beads. Pipet sample directly on top of the beads at the bottom of the tube to ensure immediate mixing.
9. Tighten the cap on the vial. Seal the top of the vial with parafilm to avoid leakage. Incubate on a rotator for 30 minutes at RT at maximum speed.
10. Allow the magnetic beads to settle then remove the supernatant taking care not to remove any beads. The depleted peptide solution may be frozen at -80° C and saved for other experiments.
11. Wash the beads by adding 1 mL of Wash Buffer, mix by inverting the tube until the beads are completely resuspended. Allow the beads to settle, and remove supernatant with a P-1000 micropipettor. Repeat the wash two more times and remove any remaining supernatant.
12. Elute the phospho-peptides from the beads by adding 50 µL of Elution Buffer (50% acetonitrile, 2.5% ammonia). Resuspend the beads by tapping the bottom of the tube, and allow the beads to settle.
13. Rinse a new microcentrifuge tube with 0.5 mL acetonitrile to remove any contaminants, vortex and discard the rinse. Transfer the supernatant containing the eluted phospho-peptides to the rinsed tube.
14. Add 40 µL of 20% TFA to acidify the eluate.
15. Repeat the elution step, and combine the resulting eluates.
16. Dry the eluted phospho-peptides in a Speed-Vac (about 3 hours). Reconstitute the dried phospho-peptides with 40 uL of 0.1% TFA.

IV. Concentration and Purification of Peptides for LC-MS Analysis on C18 tip

NOTE: We recognize there are many other routine methods for concentrating peptides using commercial products such as ZipTip® and C18 tips (see below) that have been optimized for peptide desalting/concentration. Regardless of the particular method, we recommend that the method of choice be optimized for recovery and be amenable for peptide loading capacities of at least 10 µg.

C18 tips: Thermo Scientific, part number SP201

ZipTip®: EMD Millipore, catalog number ZTC18S960

A. Solutions and Reagents

NOTE: Prepare solutions with Burdick and Jackson water or other HPLC grade water. Organic solvents (trifluoroacetic acid, acetonitrile) should be of the highest grade. PierceTM Trifluoroacetic Acid (TFA), Sequencing grade (Thermo Scientific, 28903) and PierceTM Acetonitrile (ACN), LC-MS Grade (Thermo Scientific, 51101) are recommended.

1. **Solvent C (0.1% trifluoroacetic acid, 50% acetonitrile):** add 0.1 ml trifluoroacetic acid to 40 ml HPLC water, then add 50 ml acetonitrile, adjust the final volume to 100 ml with HPLC water.
2. **Solvent D (0.1% trifluoroacetic acid):** add 0.1 ml trifluoroacetic acid to 50 ml HPLC water, adjust the final volume to 100 ml with HPLC water.
3. **Solvent E (0.1% trifluoroacetic acid, 40% acetonitrile):** add 0.1 ml trifluoroacetic acid to 30 ml HPLC water, then add 40 ml acetonitrile, adjust the final volume to 100 ml with HPLC water.

NOTE: Organic solvents are volatile. Tubes containing small volumes of these solutions should be prepared immediately before use and should be kept capped as much as possible, because the organic components evaporate quickly.

B. Procedure

1. Equilibrate the C18 tip by passing 50 µl of Solvent C through (once) followed by 50 µl of Solvent D two times.
2. Load sample by passing the phospho-peptide solution through the C18 tip. Wash the tube containing the phospho-peptide solution with 40 uL Solvent D and apply the rinse to the conditioned C18 tip.
3. Wash the C18 tip by passing 55 µl of Solvent D through two times.
4. Elute peptides off the C18 tip by passing 10 µl of Solvent E through two times. Collect the eluate in an Eppendorf tube, HPLC autosampler vial insert, or 96-well plate. Pool the two resulting eluates.
5. Dry down the C18 tip eluate in a vacuum concentrator (Speed-Vac) and redissolve the peptides in an appropriate solvent for LC-MS analysis such as 5% acetonitrile, 0.1% TFA.

Safety Data Sheet (SDS) According to the OSHA Hazard Communication Standard 29 CFR 1910.1200

Issuing Date: 2016-08-19 **Revision Date:** 2016-08-05 **Version:** 1

SECTION 1. Identification

Product identifier

Product number 20432
Product name PTMScan® Phospho-Enrichment IMAC Fe-NTA Magnetic Beads

Recommended use of the chemical and restrictions on use

Identified uses This product is intended for research purposes only.
Uses advised against This product is not intended for use in diagnostic procedures or therapeutics. This product is not intended for use in humans or animals.

Manufacturer, importer, supplier

Manufacturer address Cell Signaling Technology, Inc.
3 Trask Lane
Danvers, MA 01923
United States
TEL: +1 978 867 2300
FAX: +1 978 867 2400
Website www.cellsignal.com
Email address support@cellsignal.com
Emergency telephone number In case of emergency call CHEMTREC 1-800-424-9300

SECTION 2. Hazard(s) identification

Classification

This substance/mixture is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Flammable liquids	Category 3

GHS Label elements, including precautionary statements

Signal Word
Warning

Hazard statement(s)
Flammable liquid and vapor.
.

Precautionary Statement(s)
Wear protective gloves/protective clothing/eye protection/face protection. Keep away from heat/sparks/open flames/hot surfaces.

— No smoking. Keep container tightly closed. Ground/Bond container and receiving equipment. Use explosion-proof electrical/ventilating/lighting/equipment. Use only non-sparking tools. Take precautionary measures against static discharge.
IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.
In case of fire: Use CO2, dry chemical, or foam for extinction.
Store in a well-ventilated place. Keep cool.
Dispose of contents/container to an approved waste disposal plant.

Supplementary Hazard Information

No information available.
Hazards not otherwise classified (HNOC)
Not applicable.

SECTION 3. Composition/information on ingredients

Chemical Name	CAS No	Weight %
ethanol	64-17-5	10- <=24
Fe-NTA MagBeads, Fe-NTA Agarose	NONE	0.1 - <=2.5

SECTION 4. First-aid measures

Eye contact Immediately flush with plenty of water. After initial flushing, remove any contact lenses and continue flushing for at least 15 minutes. Get medical attention if irritation persists.
Skin contact Wash skin with soap and water. Get medical attention if irritation develops and persists.
Inhalation IF INHALED: Remove to fresh air and keep at rest in a position comfortable for breathing. Get medical attention immediately if symptoms occur.
Ingestion Clean mouth with water and afterwards drink plenty of water. Do NOT induce vomiting. Get medical attention.

Most important symptoms and effects, both acute and delayed

Symptoms of overexposure may be headache, dizziness, tiredness, nausea and vomiting

Indication of any immediate medical attention and special treatment needed

Treat symptomatically.

Advice for emergency responders

General advice For further assistance, contact your local Poison Control Center.
Protection of first-aiders Ensure that medical personnel are aware of the material(s) involved, and take precautions to protect themselves.

SECTION 5. Fire-fighting measures

Extinguishing media

Suitable Extinguishing Media Carbon dioxide (CO_2). Dry chemical. Alcohol-resistant foam.
Unsuitable Extinguishing Media CAUTION: Use of water spray when fighting fire may be inefficient.

Specific hazards arising from the chemical
Flammable.

Explosion Data

Sensitivity to Mechanical Impact None.

Sensitivity to Static Discharge Yes.

Protective Equipment and Precautions for Firefighters

As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear.

SECTION 6. Accidental release measures

Personal precautions, protective equipment and emergency procedures

For non-emergency personnel Evacuate personnel to safe areas. Ensure adequate ventilation. Remove all sources of ignition. Take precautionary measures against static discharges. All equipment used when handling the product must be grounded. Do not touch damaged containers or spilled material unless wearing appropriate protective clothing. Use personal protective equipment.
Other information No information available.

Environmental precautions

Beware of vapors accumulating to form explosive concentrations. Vapors can accumulate in low areas.

Methods and material for containment and cleaning up

Methods for containment Prevent further leakage or spillage if safe to do so.
Methods for cleaning up Soak up with inert absorbent material (e.g. sand, silica gel, acid binder, universal binder, sawdust). Pick up and transfer to properly labeled containers. Clean contaminated surface thoroughly.

SECTION 7. Handling and storage

Precautions for safe handling

Avoid contact with skin, eyes and clothing. Do not breathe vapors or spray mist. Wear personal protective equipment. Remove and wash contaminated clothing before re-use. Keep away from open flames, hot surfaces and sources of ignition. Take precautionary measures against static discharges. Use only in an area containing flame proof equipment.

Conditions for safe storage, including any incompatibilities

Technical measures/Storage conditions Keep container tightly closed in a dry and well-ventilated place. Keep away from open flames, hot surfaces and sources of ignition.
Packaging material No information available.
Incompatible products None known based on information supplied.

SECTION 8. Exposure controls/personal protection

Control parameters

Chemical Name	ACGIH TLV	OSHA PEL	NIOSH REL
ethanol	STEL 1000 ppm	TWA : 1000 ppm TWA : 1900 mg/m³	IDLH : 3300 ppm TWA : 1000 ppm TWA : 1900 mg/m³

Appropriate engineering controls

Showers, eyewash stations, and ventilation systems.

Individual protection measures, such as personal protective equipment

Personal protective equipment (PPE) needs to be selected depending on the implemented engineering controls, frequency/duration of work activities and the concentrations of the hazardous substance.

Eye/face protection If splashes are likely to occur, wear:. Tightly fitting safety goggles.
Skin and body protection Wear protective gloves/clothing. Chemical resistant apron. Lightweight protective clothing. Antistatic boots.
Respiratory protection If exposure limits are exceeded or irritation is experienced, NIOSH/MSHA approved respiratory protection should be worn. Positive-pressure supplied air respirators may be required for high airborne contaminant concentrations. Respiratory protection must be provided in accordance with current local regulations.
Hygiene measures Handle in accordance with good industrial hygiene and safety practice. Keep away from food, drink and animal feeding stuffs. Do not eat, drink or smoke when using this product. Remove and wash contaminated clothing and gloves, including the inside, before re-use. Provide regular cleaning of equipment, work area and clothing. Wash hands before breaks and immediately after handling the product.

SECTION 9. Physical and chemical properties

Information on basic physical and chemical properties

Physical state	Liquid		
Appearance	Suspension	**Color**	Clear
Odor	Characteristic	**Odor Threshold**	No information available

Property	**Values**	**Remarks Method**
pH	6.5	@ 20 °C
Melting point/freezing point		
Initial boiling point and boiling range		
Flash point	approx. 36 (23 - <=60) °C (based on components)	
Evaporation rate	No information available	
Flammability (solid, gas)	No information available	
Upper flammability limit		
Upper flammability limit	15.0%	
Lower flammability limit	3.5%	
Vapor pressure	59 hPa	44 mmHg
Vapor density	No information available	
Relative density	No information available	
Solubility	dispersible	
Solubility in other solvents	No information available.	
Partition coefficient: n-octanol/water	No information available	
Autoignition temperature	425 °C	
Decomposition temperature	No information available	
Viscosity	No information available	
Viscosity, dynamic	No information available	
Explosive properties	No information available	
Oxidizing properties	No information available	

Other information

Softening point	No information available
Molecular Weight	No information available
VOC content	No information available
Density	No information available.
Bulk Density VALUE	No information available.

SECTION 10. Stability and reactivity

Reactivity

No information available.

Chemical stability

Stable under recommended storage conditions.

Possibility of hazardous reactions

Hazardous reactions	None under normal processing.
Hazardous polymerization	None under normal processing.

Conditions to Avoid

Heat, flames and sparks. Extremes of temperature and direct sunlight.

Incompatible Materials

None known based on information supplied.

Hazardous Decomposition Products

None known based on information supplied.

SECTION 11. Toxicological information

Information on likely routes of exposure

Inhalation	There is no data available for this product. Avoid breathing vapors or mists. Vapours may irritate throat and respiratory system.
Eye contact	There is no data available for this product. Avoid contact with eyes. Contact with eyes may cause irritation.
Skin contact	There is no data available for this product. Avoid contact with skin. Prolonged or repeated contact may dry skin and cause irritation.
Ingestion	There is no data available for this product. Ingestion may cause gastrointestinal irritation, nausea, vomiting and diarrhea. May cause central nervous system depression.

Information on toxicological effects

This material should only be handled by, or under the close supervision of, those properly qualified in the handling and use of potentially hazardous chemicals. It should be borne in mind that the toxocological and physiological properties of this compound is not well defined.

Chemical Name	LD50 Oral	LD50 Dermal	LC50 Inhalation
ethanol	7060 (Rat)	-	-

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Symptoms	Symptoms of overexposure may be headache, dizziness, tiredness, nausea and vomiting.
Sensitization	No information available.
Mutagenic effects	No information available.
Carcinogenicity	Contains no ingredient listed as a carcinogen. Ethanol has been shown to be carcinogenic in long-term studies only when consumed as alcoholic beverage. Contains a suspected carcinogen. However, this component is not at a concentration considered for classification. Based on available data, the classification criteria are not met.
Reproductive toxicity	No information available.
STOT - single exposure	No information available.
STOT - repeated exposure	No information available.
Neurological effects	No information available.
Aspiration Hazard	No information available.

SECTION 12. Ecological information

Ecotoxicity

Harmful to aquatic life

Chemical Name	Toxicity to algae	Toxicity to fish	Toxicity to daphnia and other aquatic invertebrates
ethanol	-	LC50 100 mg/L (Pimephales promelas) 96 h LC50 13400 - 15100 mg/L (Pimephales promelas) 96 h LC50 12.0 - 16.0 mL/L (Oncorhynchus mykiss) 96 h	EC50 2 mg/L (Daphnia magna) 48 h EC50 10800 mg/L (Daphnia magna) 24 h LC50 9268 - 14221 mg/L (Daphnia magna) 48 h

Persistence and degradability	No information available.
Bioaccumulation	No information available.
Mobility	No information available

Chemical Name	Octanol-Water Partition Coefficient
ethanol	-0.32

Other adverse effects

No information available.

SECTION 13. Disposal considerations

Waste Disposal Methods

Dispose of in accordance with all applicable national environmental laws and regulations.

Disposal considerations

Do not empty into drains; dispose of this material and its container in a safe way.

SECTION 14. Transport information

This material is not subject to regulation as a hazardous material for shipping.

SECTION 15. Regulatory information

North American Inventory Listing

Chemical Name	TSCA 8(b)	TSCA 12(b)	DSL	NDSL
ethanol	Listed	Not Listed	Listed	Not Listed

Canadian Workplace Hazardous Materials Information System (WHMIS) Classification

	Class B2 - Flammable Liquids

SARA 313

Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 (SARA). This product does not contain any chemicals which are subject to the reporting requirements of the Act and Title 40 of the Code of Federal Regulations, Part 372.

SARA 311/312 Hazard Categories

Acute Health Hazard	No
Chronic Health Hazard	No
Fire Hazard	Yes
Sudden Release of Pressure Hazard	No

Reactive Hazard	No

Clean Water Act

This product does not contain any substances regulated as pollutants pursuant to the Clean Water Act (40 CFR 122.21 and 40 CFR 122.42).

CERCLA

This material, as supplied, does not contain any substances regulated as hazardous substances under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302) or the Superfund Amendments and Reauthorization Act (SARA) (40 CFR 355). There may be specific reporting requirements at the local, regional, or state level pertaining to releases of this material.

California Proposition 65

Ethyl alcohol is only a considered a Proposition 65 developmental hazard when it is ingested as an alcoholic beverage.

Chemical Name	California Prop. 65
ethanol	Carcinogen Developmental

U.S. State Right-to-Know Regulations

Chemical Name	New Jersey	Massachusetts	Pennsylvania
ethanol	Listed	Listed	Listed

U.S. FIFRA Label Information

This product does not contain any substances regulated as pesticides.

US Commerce Department - Export Administration Regulations Information

This product does not contain any substances regulated under the Chemical Weapons Convention (CWC).

U.S. Drug Enforcement Administration Information

This product does not contain any substances regulated under the DEA.

SECTION 16. Other information

Revision Date: 2016-08-05

Disclaimer

The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text.

End of Safety Data Sheet