# **Exploring the IMCSTIDS® benefits of IMCSTIDS®** AUTOMATED PHOSPHOPEPTIDE ENRICHMENT

## PRODUCT OVERVIEW

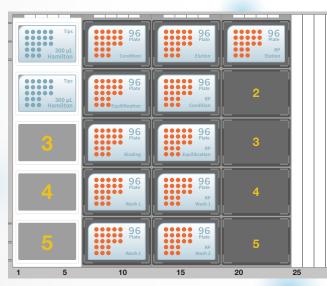
IMCStips use a patented dispersive resin technology to enrich phosphorylated proteins and peptides. Tyrosine is a crucial target for phosphorylation in mediating cellular signaling events linked to carcinogenesis. The lower abundant phosphotyrosine (pTyr) is difficult to achieve using zirconium dioxide (ZrO<sub>2</sub>) or titanium (PolyTi<sup>™</sup>) chemistries, as these chemistries enrich more abundant phosphorylated serines (pSer) and threonines (pThr). An improved enrichment of pTyr peptides is achieved with immunoaffinity enrichment workflow that couples anti-pTyr antibodies to either Protein A or Streptavidin beads in IMCStips.

- HIGH SPECIFICITY—over 90% specificity for pTyr within enriched phosphopeptides
- AUTOMATED—hands-off sample processing
- EASY TO USE—ready-to-run workflow and robotic script available
- FLEXIBLE—available in multiple tips sizes with multiple resin amounts

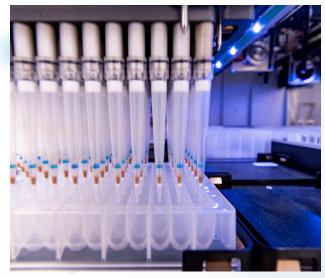
### FULLY AUTOMATED METHODS AND CUSTOM SCRIPTS AVAILABLE

IMCS offers fully automated sample preparation methods for pTyr enrichment IMCStips. Methods have been optimized and are ready for use in your process.

- 96 samples in 30 minutes
- We will send a software script package file for Hamilton systems with your tips
- Our R&D team can help develop a customized workflow
- We offer onsite confidential method development



Deck layout on Hamilton Microlab STAR liquid handling system for automated method for desalting and phosphopeptide enrichment.



HAMILTON

Barrier

Disperser

Loose Resin

Frit

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CO-RE Technology

Example of IMCStips on Hamilton 96 CO-RE head.



## IMPLEMENTATION IS EASY WITH IMCStips®

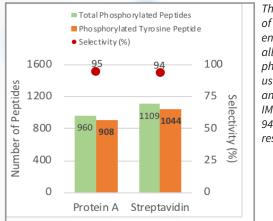
- Software scripts for Hamilton Workstations
- Fully developed narratives guide you through each step
- Templated processes allow for customized workflows
- On-site technical support



## HIGH PERFORMANCE AND CONSISTENCY

#### **Sequential purification of phosphopeptides:** 1. Primary enrichment of pTyr using automated workflow with pY1000 or 4G10 anti-pTyr antibody

2. Secondary enrichment of phosphopeptides using ZrO<sub>2</sub> or PolyTi<sup>™</sup> for enrichment and analysis of pSer/ pThr targets.



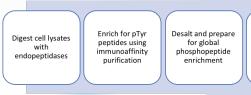
The specificity of pTyr peptide enrichment from all identified phosphopeptides using Protein A and Streptavidin IMCStips were 94.6% and 94.1 %, respectively.

pTyr enrichment was performed using either pY1000 antibody (Cell Signaling Technology), 4G10 antibody (MilliporeSigma), or 1:1 mix of these two antibodies with streptavidin IMCStips. Subsequent comprehensive phosphorylated peptides enrichment of pTyr immunoaffinity flowthroughs was performed using ZrO<sub>2</sub> and PolyTi<sup>™</sup> IMCStips. While there was no difference of the enrichment efficiency of these two resins from fresh digests, PolyTi<sup>™</sup> IMCStips identified > 1.7 times more phosphorylated peptides than ZrO<sub>2</sub> IMCStips.

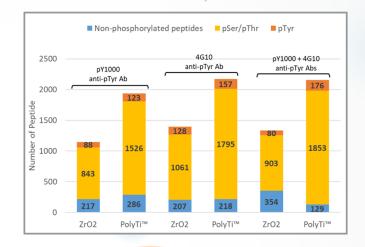
integrated micro-chrom

Product Description	Catalog Number Rack of 8	Catalog Number Rack of 96
PolyTi™ 5 mg resin-300 μL tips	04T-H3R73-1-5-8	04T-H3R73-1-5-96
ZrO <sub>2</sub> 5 mg resin-300 μL tips	04T-H3R68-1-5-8	04T-H3R68-1-5-96
Protein A 5 μL resin - 300 μL tips	04T-H3R80-1-5-8	04T-H3R80-1-5-96
Streptavidin 3 μL resin- 300 μL tips	04T-H3R85-1-3-8	04T-H3R85-1-3-96

#### Workflow diagram



Further enrich remaining phosphopeptides from the flowthrough for enhanced coverage





The overlap of identified phosphorylated tyrosine (pTyr) protein using either streptavidin or protein A IMCStips was 78.4%.

<b>ORDER A COMPLIMENTARY SAMPLE TODAY</b>
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- Call, email or visit www.imcstips.com to order Phosphopeptide Enrichment IMCStips<sup>®</sup>
- Schedule a consultation with an IMCS technical sales representative for more information.