



ZipChip<sup>™</sup> System for Selected Thermo Scientific<sup>™</sup> Mass Spectrometers -- for CE/ESI-MS Analyses

The world leader in serving science

### Content





## What is the ZipChip System?

- The ZipChip system uses integrated microfluidic technology to prepare, separate samples by capillary electrophoresis (CE), and then electrospray (ESI) analytes directly into a mass spectrometer (MS)
- It is composed of the ZipChip interface and the microfluidic chip
- ZipChip Interface directly mounts onto the front end of a mass spectrometer
- The CE separation and ESI occur on the microfluidic chip
- ZipChip system is compatible with a broad range of biomatrices such as growth media, cell lysates, blood, plasma, and urine

		PIN 810-00140 ZipChip <sup>M</sup> HR HR000616	
	+15 KV	EC Pump Electroosmotic flow (EOF)	
	ZipChip HS	ZipChip HR	
Separation channel length (cm)	10	22	
Flowrate (nL/min)	150	150	
Maximum # of injections per chip	125	125	
On Chip De-salting capability	Yes	Yes	
Integrated ESI Emitter	Yes	Yes	
EEPROMS (recognize chip type and track usage)	Yes	Yes	
Recommended use	Small molecules or simple sample mixture	Big molecules or complex sample mixture	
Typical analysis time	Up to 3 min	Up to 15 min	

# Why the ZipChip System?



- Capillary Electrophoresis (CE) has unique advantages for certain analyses where traditional LC has its limitation
  - > Separation of charge variants of intact antibodies/proteins
  - Effective separation of monosaccharides, glycans, and glycopeptides in a MS compatible condition
  - Separation of very hydrophilic molecules
  - > Separation of amino acid isomers
  - Analyses of samples in high salt condition
- Other CE instruments that can be coupled with MS are bulky with cumbersome operations and take longer analysis time
- They are not specifically designed to be coupled with MS therefore requires additional interface
- There is a lack of convenient CE/ESI device that offers fast and good CE separation for mass spectrometry analysis
- ZipChip is the ONLY commercially available portable and integrated CE/ESI source designed for mass spectrometers

Fast CE separation • Nano Spray Sensitivity • HRAM Mass Spectrometry

#### Unique Values and Common Applications

#### **Unique Values**

- The only commercially available integrated and portable CE/ESI interface for MS
- Offers extremely rapid CE separations, nano-spray level sensitivity, and HRAM mass spectrometry in one platform
- Requires minimal sample preparation
  with on-chip desalting capability
- Consumes only picograms to nanograms of sample per analysis

#### **Common Applications (not a full list)**

- Intact mAb and ADC/biotherapeutics characterization
  - ✓ Native condition
  - ✓ Denatured condition
- mAb subunit analyses
- Glycomics and glycoproteomics
- Peptide mapping
- Metabolomics



### **Comprehensive Portfolio**











#### ZipChip Interface

#### ZipChip Autosampler

#### DryDock

#### ZipChips

#### ZipChip Assay Kits

- Compatible with all Thermo Scientific<sup>™</sup> Exactive, Q Exactive Orbitrap MS, and LTQ Orbitrap MS instruments
- Data collection, processing and reporting through Thermo Scientific<sup>™</sup> Xcalibur and BioPharma Finder software
- Two versions: Autosampler operation version and manual operation version

- Required to have for ZipChip Interface autosampler version
- Fully automated and controlled by the ZipChip software
- Conduct chip priming, sample and BGE loading, and chip flushing
- Compatible with both 48vial plate and 96-well plate

- A simply drying accessary
- No electrical component
- Utilize the MS gas to push air through the ZipChip channels removing residual fluid
  - Dry 1 chip each time

- Disposable chips
- Good for up to 125
  injections
- Two types: HR chip and HS chip
- Each chip is a single piece of glass similar as a microscope slide, housed in • PEEK polymer
- Microfluidic channel and electrospray is integrated into both types of chips
- 5 chips per pack

- 3 types of pre-made assay kits are designed for intact antibody, peptides, and metabolites analyses, respectively
- Each box of assay kit contains 5 bottles of BGE and 5 bottles of sample diluents.
- Individual bottles can be directly loaded into ZipChip autosampler
- Each individual bottle contains enough volume for 100 runs
- Each box is good for 500 runs



## ZipChip-MS Analysis Workflow



Select proper assay kit and ZipChip for your experiments

Simple Sample Prep



Set up sequence and collect CE-MS data

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# Anatomy of ZipChip





## Sample Injection





10  $\mu$ M rhodamine-6G in BGE + 100 mM ammonium acetate

### Sample Separation

- High voltage applied to Wells 2 and 4
- HV1 and HV2 determine field strength

 $Field Strength = \frac{HV1 - HV2}{Channel Length}$ 

• Field strength drives the ZipChip separation



 $\mu_{EP} = \frac{q}{6\pi\eta a} \begin{cases} q - charge \\ \eta - viscosity \\ a - hydrodynamic radius \end{cases}$ 



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## The ZipChip System with Thermo Scientific<sup>™</sup> Q Exactive<sup>™</sup> BioPharma Platform



BioPharma Option is available for Q Exactive Plus, Q Exactive HF and Q Exactive HF-X mass spectrometers

### Intact mAb - Trastuzumab Analysis in HMR mode

The ZipChip system coupled with the Q Exactive BioPharma platform can quickly analyze intact mAbs in native, partially denatured, and fully denatured conditions to support biotherapeutics characterizations under a diverse range of conditions

- CE/ESI-MS analysis can be completed within 3 minutes
- High resolution accurate mass spectra in intact native, partially denatured, and fully denatured states on the Q Exactive Plus/HF/HF-X MS with BioPharma option are confidently achieved
- Sample consumption can be as low as pico grams to nano grams
- Major glycoforms are identified by BioPharma Finder software

MS data was acquired on a Q Exactive HF-X MS with BioPharma Option CE separation was achieved on ZipChip HR

with 10mM Ammonium Acetate and 10% Isopropanol (native), Intact Antibody Assay kit (partially denatured), or Peptides Assay Kit (fully denatured)





#### Intact mAb - Infliximab Analysis in HMR mode

The ZipChip system coupled with the Q Exactive MS BioPharma platform is unique and powerful to separate and identify different intact antibody charge variants in native and denatured conditions

- Baseline separation of intact mAb charge variants resulting from different levels of Lys-clipping can be achieved within three minutes by ZipChip system
- High resolution accurate mass spectra of all lysine variants are confidently detected on Q Exactive Plus/HF/HF-X MS with BioPharma option
- Three major glycoforms from each of the three lysine variants are identified by BioPharma Finder with mass accuracies better than 10 ppm

MS data was acquired on a Q Exactive HF-X MS with BioPharma Option CE separation was achieved on ZipChip HR with 10mM Ammonium Acetate in 10% Isopropanol as BGE

## Intact NIST mAb Analysis in HMR Mode

The rapid separation and accurate identification of highly differently abundant charge variants can also be consistently achieved by the ZipChip system and Q Exactive BioPharma platform

- Near baseline separation of intact NIST mAb charge variants with abundance ranging over 2 orders of magnitudes can be achieved by the ZipChip system
- High resolution accurate mass data of each Lysine variant is confidently obtained on Q Exactive Plus/HF/HF-X MS
- Glycoform with abundance as low as . 0.16% of the base peak can be detected and identified
- All 5 major glycoforms from each of the three different Lysine variants are identified by BioPharma Finder



with Intact Antibody Assay Kit



4789.87

4800

4810.97

50-

0

4600

4640.21

4971.14

5000

m/z

5142.36

5200

5308.79

5400

4960.26

4960

m/z

4965.69

4980

4944.11

4940

80-

60-

40-

20-0-

4920

# Antibody-Drug Conjugate (ADC) Analysis in HMR Mode

Heterogenetic ADCs can be successfully characterized within 1 minute without sample pre-treatment by the ZipChip system and Q Exactive BioPharma platform, and the powerful BioPharma Finder software

- Powerful sliding window capability enabled by BioPharma Finder software
- All of the different forms of Trastuzumab Emtansine can be analyzed in less than 40 seconds with only 3 nanograms of sample injected
- No sample pre-treatment required
- The calculated average DAR value is consistent with previous published data

MS data was acquired on a Q Exactive HF with BioPharma Option

CE separation was achieved on ZipChip HR with Intact Antibody Assay Kit



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- Separation of infliximab mAb . subunits can be achieved in 3 minutes by ZipChip system
- The sliding window method combined . with Thermo Scientific<sup>™</sup> Xtract<sup>™</sup> deconvolution algorithm in **BioPharma Finder software enables** monoisotopic mass determination of each subunit
- Lysine variants and their major • glycoforms of the subunits can be identified by BioPharma Finder software

MS data was acquired on a Q Exactive HF-X MS with BioPharma Option CE separation was achieved on ZipChip HR with Peptides Assay Kit



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## Peptide Mapping Analysis in Standard Mode

The combination of ZipChip sample separation, Q Exactive Plus/HF/HF-X MS platform produced HRAM MS and MS/MS spectra, and BioPharma Finder software enables fast and accurate peptide identification

- Plug and play ZipChip delivers stable nano spray and nano spray level sensitivity
- CE-MS/MS analysis can be completed in 10 minutes
- Only a few nanograms of sample are sufficient for the analysis
- 98% sequence coverage based on MS/MS data for the light chain and heavy chain is confidently achieved

MS data was acquired on a Q Exactive Plus MS with BioPharma Option

CE separation was achieved on ZipChip HR with Peptides Assay kit



Proteins	Number of MS Peaks	MS Peak Area	Sequence Coverage	Abundance
NSIT mAb light chain	141	26.4%	100.0%	41.67%
NIST mAb heavy chain	339	60.5%	97.6%	56.35%
Unidentified	1441	12.6%		

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### Complete 20 Amino Acids Analysis

Extremely rapid separation and accurate identification of all 20 amino acid mix can be confidently achieved by the ZipChip system and Thermo Scientific Q Exactive /Focus/Plus/HF/HF-X MS platform

- Separation of 20 amino acids can be completed in 2 minutes by the ZipChip system
- Isomers, Leucine(L) and Isoleucine(I), can be baseline separated
- Co-elueted amino acids can be identified with HRAM mass spectrometry
- Femto grams level of sample is sufficient for each analysis

MS data was acquired on a Q Exactive MS

CE separation was achieved on ZipChip HS with Metabolites Assay kit



## Linear Dynamic Range Test of Arginine and Valine

Minimum sample loading of 40 attomole Arginine and Valine can be achieved by the ZipChip system and Thermo Scientific Q Exactive /Focus/Plus/HF/HF-X MS platform

- Arginine and Valine calibration curves provide good linearity over the range of 10 nM to 10  $\mu M$
- Lowest sample injection amount on the linear curve is 40 attomoles (4nL of 10 nM sample injected)



#### Arginine (R) 10nM~10µM

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MS data was acquired on a Q Exactive MS

CE separation was achieved on ZipChip HS with Metabolites Assay kit

### Quick Analysis of Amino Acids in Gibco™ DMEM Cell Culture Media

The ZipChip system and Thermo Scientific Q Exactive /Focus/Plus/HF/HF-X MS platform can rapidly separate and identify the amino acids presented in cell culture media

- Sample can be directly analyzed after simple filtration and dilution
- On chip desalting capability allows direct analysis of samples in high salt condition without pre-treatment
- Separation can be completed in 2 minutes by the ZipChip system
- All 15 amino acids presented in the cell culture media are identified and results match up with the aa composition indicated in the commercial sample spec sheet

MS data was acquired on a Q Exactive MS

CE separation was achieved on ZipChip HS with Metabolites Assay kit



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#### Calibration Curve of Internal Standard in Cell Culture Media

Accurate quantification of amino acids in cell culture media with stable isotope labeled internal standard can be achieved by the ZipChip system and Thermo Scientific Q Exactive /Focus/Plus/HF/HF-X platform

- Deuterated Valine standard, Valined8, was spiked into the crude cell culture media
- High linearity calibration curve with concentration range from 50nM to 10μM can be established.

Val-d8  $D_3C D O$ Concentration range: 50 nM to 10µM  $D_3C$ OH D NH<sub>2</sub> 3.E+08 2,E+08 y = 2E + 07x + 484283 $R^2 = 0.9996$ Signal Intensity 2,E+08 1,E+08 5.E+07 0.E+00 2 6 8 10 12 4 Λ Concentration (µM)

MS data was acquired on a Q Exactive Classic MS

CE separation was achieved on ZipChip HS with Metabolites Assay kit



## Carryover Test by Monitoring Arginine

#### The ZipChip system has extremely low carryover

- Blank injections interleaved between . 10 µM AA mix injections
- Extracted ion electropherogram of • Arginine from sample injection and blank injection were used to calculate the carryover
- Observed carryover of Arginine is • only 0.054% or less

MS data was acquired on a Q Exactive Classic MS



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Assay kit

## Migration Time Reproducibility Test Using Arginine (R) Standard

Operation of the ZipChip system through ZipChip autosampler can achieve good reproducibility

- The migration time of Arginine was monitored throughout 100 runs; the overall RSD value of the migration time is 1.85%
- It takes 5-10 injections for the system to get stabilized
- First 50 injections are from the same sample vial; the RSD value of the migration time is 1.76%; the RSD value dropped to 0.54% if excludes the first 7 injections
- Last 50 injections are from 50 different sample wells; the RSD value of the migration time is only 0.84%

MS data was acquired on a Q Exactive MS

CE separation was achieved on ZipChip HS with Metabolites Assay kit



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#### **Available Resources**

#### **CE/ESI-MS Brochure**

Hardware Spec Sheets

Consumable Spec sheet

**Application Note** 

www.thermofisher.com/zipchip

#### 908devices.com/products/zipchip





Profiling Of Clinically Relevant Proteoforms In Human Tears Using Chip-Based Capillary Electrophoresis Coupled To Mass Spectrometry Caniel Lopes-Ferrer', Romain Huguet', Andreas Krupke', Chien-Haan Chen', A Purpose: Denorances the adential for policing acceptones in tags a second second state annotations of the cases of new shoeld exclusion case, privat adenticities further addition to exclusion case, privat adenticities further addition to the cases of the case of the case of the case of the cases of the cases of the case of the case of the cases of the cases of the cases of the cases of the case of the cases of the cas Solide till allows to rouge politing and reprinting of same in protections in tests in stress 5 million come up the school of a ing Jacon A. Barlay, and Michael Maintenismin Drug Conjugate Varian Price stress 1/2,2/2,1 (in sevenuin spinor spinor her 1, 4) for each an energy many the sevenuin sector of the spinor spinor distance of the spinor many spinor spin

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#### ZipChip Interface for Mass Spectrometry



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#### ZipChip Interface-seamless workflow for MS analysis of biological samples

Integrate capillary electrophoresis (CE) and electrospray ionization (ESI) into a single microfluidic device to rapidly prepare, separate, and electrospray biological samples directly into your Thermo Scientific mass spectrometer. The portable size ZipChip™ Interface directly mounts onto select models of Thermo Scientific mass spectrometers, and creates a seamless CE-MS workflow that offers fast CE separation, nano-spray level sensitivity, and HRAM spectrometry for the characterization of intact proteins, antibody drug conjugates (ADCs), antibody subunits, peptides, and metabolites

The ZinChip Interface is a Class 1 laser product in compliance with 21 CER 1040.10 and 1040.11 except for deviations pursuant to laser notice No. 50





ZipChip

#### **Empowering Traditional Mass Spec**

The ZipChip™ separations platform uses integrated microfluidic technology to prepare, separate and electrospray biological samples directly into traditional mass spectrometers (MS). In less than 3 minute per sample, the cost-effective ZipChip system enables analysis of a broad range of matrices from growth media to cell lysates, blood, plasma, urine, and biopharma products. Each chip provides answers on analytes from small molecules up to intact proteins, antibodies and antibody drug conjugates (ADCs). This platform provides better separation quality than all with full MS identification behind every separations peak.





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# Ordering Information

Product	P/N	
Hardware		
ZipChip Interface (Autosampler version)	00950-01-00492	
ZipChip Interface (Manual version)	00950-01-00493	
ZipChip Autosampler	00950-01-00494	
DryDock	00950-01-00518	
Consumables		
Installation Test Kit	00950-01-00510	
ZipChip HS	00950-01-00498	
ZipChip HR	00950-01-00499	
ZipChip Metabolites Assay Kit	00950-01-00500	
ZipChip Peptides Assay Kit	00950-01-00501	
ZipChip Intact Antibody Assay Kit	00950-01-00502	



Thank you!

Any Questions?

