ZipChip Sample Guide

The ZipChip™ system uses microfluidic zone electrophoresis to separate molecules based on their charge and size. For many applications ZipChip separations can achieve better results but may require different considerations to be made with regard to sample type, and sample prep, in order to be successful.

Is this application suitable for ZipChip Analysis?

1. Do your analytes have the proper charge?

For all applications except for oligonucleotide analysis, only molecules that are **positively charged at the pH of the background electrolyte (BGE)** can be analyzed with the ZipChip system. The charge versus pH of most small molecules can be estimated using the excellent website, chemicalize.org. The pH values for the ZipChip kit BGEs are listed below:

BGE Kit	pH
Metabolite	2.2
Peptide	2.4
Intact	3.3
Native	5.5
Charge Variant TOF	5.5

For analysis of intact proteins, ensure that the **isoelectric point of the protein is greater than the pH of the BGE** under consideration.

2. Are your analytes concentrated enough?

Think in terms of sample concentration, not total amount loaded on the chip. ZipChip separation is sensitive to the concentration of each analyte in the mixture, not the total amount or concentration of all of the analytes combined. The following table lists approximate limits of detection for ZipChip analysis of three classes of analytes.

Limit of Detection†	
Metabolites	1-10 nM
Peptides	1-10 nM*
Intact Antibody	0.001 mg/mL
Native Antibody	0.001 mg/mL

†Limits of detection are approximating for full scan MS on a QExactive. LODs will vary based on the MS instrument and MS method used.
* Concentration of individual peptides, not the starting concertation of the protein

What concentration should I make my samples?

This question is straightforward for some applications, but much more complicated for others. Please refer to the ZipChip Application Protocols for application specific details. Here are a few examples:

- **Small molecule or peptide standards**: Standards should be prepared using the Sample Diluent provided in the BGE kit at a minimum of 4x dilution to a target concentration of **1 to 10 μM**.
- **Growth media analysis**: For quantitation of amino acids and biogenic amines, growth media samples can be **diluted 100x** with the Metabolite Kit sample diluent, or until the more concentrated components are just below the onset of overloading.
- **Peptide mapping of a monoclonal antibody**: The final concentration of the digested protein sample after 4-5x dilution with the Peptides kit diluent should be in the range of **0.1 to 0.5 mg/mL**.
- **Intact protein under denaturing conditions**: These samples should be diluted directly from formulation to a concentration of **0.1 to 1 mg/mL** with the BGE. Desalting is recommended if the concentration of non-volatile buffer components is greater than 10 mM after dilution. Samples with anionic and non-ionic detergents don't need any cleanup. We highly recommend using the NIST monoclonal antibody reference material (National Institute of Standards and Technology, p/n 8671) as a QC standard for intact mAb analysis.
- **Charge Variants Analaysis of mAb and related proteins**: Samples can be diluted directly into the BGE to achieve a final concentration of 0.1-1 mg/mL using the Native Kit diluent (or Charge Variant TOF Kit diluent) as long as the final concentration of non-volatile buffers is less than 10 mM. Samples containing higher than 10 mM non-volatile salt content or any amount of PBS should be buffer exchanged into the BGE.

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Overloading: For ZipChip separations, overloading causes high concentration analyte bands to broaden and become asymmetrical. It's recommended to perform a dilution study during initial method testing to understand the onset of overloading for specific analytes. This table lists the approximate concentrations where you will start to see overloading of analytes:

Onset of overloading	
Metabolites	50 μM
Peptides	50 μM
Intact Antibody	1 mg/mL

How much sample volume do I need?

ZipChip separations use microfluidic technology to analyze very small amounts of each sample (1.0 – 10 nL), but larger volumes need to be loaded into the ZipChip sample well to ensure reproducible performance. When using the ZipChip autosampler, **the minimum sample volume is 10 μL**. For manual sample introduction by pipette, the minimum sample volume is 5 μL. A sample can be analyzed multiple times before it is discarded.

What other parameters are important for sample composition?

- **The salt content of the sample is important for metabolite and peptide analyses.** These applications use an online sample focusing method which works best when the sample contains ~100 mM ammonium acetate. Ammonium is the best leading electrolyte for ZipChip separations because it has a very fast electrophoretic mobility and it is fully compatible with electrospray mass spec. Other non-volatile salts (e.g. NaCl or CH_3COONa) present in the sample are typically not a problem if they are at a concentration less than or equal to ~10 mM after 5x dilution with the sample diluent.
- **Buffers and sample additives to avoid for metabolite and peptide analyses.** High concentrations of slow migrating cations can disrupt the sample focusing process. Common examples include: **TRIS, guanidine, and TCEP**. When possible, you should choose a workflow that avoids these components. For example, protein digestions can be performed in a compatible buffer, such as ammonium bicarbonate, with compatible denaturing agents, such as RapiGest (Waters Corporation), Protease Max (Promega Corporation), or urea. For cases where samples do contain these components, we recommend buffer exchanging via solid phase extraction, dialysis, or similar before ZipChip analysis.
- **Neutral and anionic components of the sample matrix are no problem for ZipChip Analysis!** These components do not migrate down the separation channel, so they do not impact the ZipChip analysis. Examples of sample matrix components that cause problems for LC analysis but are fully compatible with ZipChip separations are: glycerol, urea, PEG, sucrose, DTT, polysorbate, and SDS.
- **It is important to consider the solubility of the analyte in both the sample matrix and the BGE.** ZipChip separations do not use heat or aggressive mobile phases to force molecules into solution. This gives ZipChip analyses significant advantages compared to LC, but you will only achieve the fantastic speed and efficiency of ZipChip separations if your analytes stay solvated throughout the analysis.
- **You generally don't need to worry about the organic content of the sample.** ZipChip separations do not rely on retention, so organic content is only important if it affects the solubility of the sample. Compatibility with a range of organic compositions means that metabolomics and peptide workflows can often be simplified for ZipChip Analysis.
- **Internal standards should be added for absolute quantitation.** You should use the same quantitation strategies for ZipChip that you use for LC/MS, for example, utilizing stable isotope amino acid standards (e.g. Cambridge Isotopes MSK-A2-1.2) enables highly accurate quantitation of amino acids in complex biological fluid samples.

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