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# Automated BioID sample preparation V.1

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**We use this protocol and it's working**

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## Abstract

We introduce an automated workflow for proximity dependent biotinylation on a liquid handler to process up to 96 samples at a time,combined with shorter liquid chromatography gradients and data-independent acquisition to increase analysis throughput and enable reproducible protein quantitation

## Materials

Sulfo-NHS-Acetate (Thermo Fisher)
- LysC (Cell Signaling)
- Trypsin (MS grade) (Thermo Fisher)
- Aprotinin (Carl Roth)
- Leupeptin (Carl Roth)
- Turbonuclease (MoBiTec GmbH)
- Trizma base (Sigma-Aldrich)
- Ammonium Bicarbonate (Sigma-Aldrich)
- HEPES (Sigma-Aldrich)
- NaCl (Sigma-Aldrich)
- EDTA (Sigma-Aldrich)
- EGTA (Sigma-Aldrich)
- Triton X-100 (Sigma-Aldrich)
- SDS (Sigma-Aldrich)
- Acetonitrile (Biosolve)
- Trifluoroacetic acid (Sigma-Aldrich)
- Methanol (Biosolve)
- Formic Acid (Sigma-Aldrich)

**Buffers**
- **PBS**
- **Lysis buffer**: 50 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton, 0.1% SDS, 1.5 µM aprotinin, 10 µM leupeptin, 250U Turbonuclease
- **Acetylation buffer:** 10 mM sulfo-NHS acetate
- **Wash buffer:** 50 mM AmBic, pH 8.3
- **Digest buffer:** 0.5 µg LysC in 50 mM AmBic
- **Elution buffer:** 10% TFA in ACN
- **Maintenance buffers:** 20% ACN

## Troubleshooting

## General concept

1 Proximity dependent biotinylation (BioID) is an important method to study protein-protein interactions in cells, for which an expanding number of applications has been proposed. The laborious and time consuming sample processing has limited project sizes so far. Here, we introduce an automated workflow on a liquid handler to process up to 96 samples at a time. The automation does not only allow higher sample numbers to be processed in parallel, but also improves reproducibility and lowers the minimal sample input. Furthermore, we combined automated sample processing with shorter liquid chromatography gradients and data-independent acquisition to increase analysis throughput and enable reproducible protein quantitation across a large number of samples. We successfully applied this workflow to optimize the detection of proteasome substrates by proximity-dependent labeling.

## Abbreviations

2 ACN - Acetonitrile,
AmBic - Ammonium Bicarbonate,
DIA – Data Independent Acquisition,
DMEM - Dulbecco's Modified Eagle Medium,
EDTA – Ethylene Diamine Tetraacetic Acid,
EGTA - (Ethylene Glycol-bis(β-aminoethyl ether)-N,N,N′,N′-Tetraacetic Acid,
FBS - Fetal Bovine Serum,
HEPES - 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid,
PBS - Phosphate-Buffered Saline,
RT - Room Temperature,
SDS - Sodium Dodecyl Sulfate,
TFA - Trifluoroacetic acid

## Reagents

3 Sulfo-NHS-Acetate (Thermo Fisher)
- LysC (Cell Signaling)
- Trypsin (MS grade) (Thermo Fisher)
- Aprotinin (Carl Roth)

- Leupeptin (Carl Roth)
- Turbonuclease (MoBiTec GmbH)
- Trizma base (Sigma-Aldrich)
- Ammonium Bicarbonate (Sigma-Aldrich)
- HEPES (Sigma-Aldrich)
- NaCl (Sigma-Aldrich)
- EDTA (Sigma-Aldrich)
- EGTA (Sigma-Aldrich)
- Triton X-100 (Sigma-Aldrich)
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**Buffers**

- **PBS**
- **Lysis buffer**: 50 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton, 0.1% SDS, 1.5 µM aprotinin, 10 µM leupeptin, 250U Turbonuclease
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- **Digest buffer:** 0.5 µg LysC in 50 mM AmBic
- **Elution buffer:** 10% TFA in ACN
- **Maintenance buffers:** 20% ACN

## Procedure

4 **Expression of fusion proteins**

4.1 Cell culture

- Day 1: Seed 1.5 Mio cells per P150 plate
- Day 2: Induce expression of BirA construct by adding Tetracycline 1:1000
  - Tetracycline stock: 1 mg/ml in Ethanol
- Day 3: Add 50 µM of Biotin (in water)
- Day 4: Collect cells

4.2 Collection of cells

- Wash cells 2x with RT PBS + Ca/Mg

- Add Trypsin and incubate 7 minutes at RT or till cells start to detach
- Add 2x DMEM with 5% FBS and pipet cells carefully off
- Spin 5 min, 500g at 4°C
- Remove supernatant and
- Resuspend cells in 10 ml cold PBS
- Count the cells (1:5 -1:10 dilution)
- Prepare tubes with 4 Mio cells each
- Spin 5 min, 500g at 4°C
- Remove supernatant
- Snap freeze samples in liquid nitrogen and store at -20°C

4.3 Preparing the Lysates

- Resuspend the pellet of 20 Mio cells in 1.25 ml of Lysis buffer in a 15ml falcon tube (scale accordingly to amount of cells)
- Lyse the cells by rotating the falcon tube for 1h, 15rpm at 4°C
- Transfer to 1.5 ml tube.
- Sonicate the sample 10× 30sec on/off in Bioruptor at 4°C

Note

No visible aggregates should be there, otherwise sonicate more

- Spin the samples 30min, 4°C, 17000g
- Keep supernatant

## Operate Agilent Bravo Automated Liquid Handling Platform

5 Starting operations

- Start Bravo AssayMAP using the pre-set "System startup" under "**Utility library"**

5.1 **2. Acetylation of cartridges** (>72 min)

- Open pre-set "On-cartridge reaction" under "**App library"**
- Add the following labware and buffers to the appropriate places:
  - #3 Equilibration & Chase Buffer: 320 µl PBS
  - #4 Reagent: 50 µl acetylation buffer

Note

Stop after equilibration and add only immediately before acetylation step

- #5 Wash Buffer 1: 300 µl BioID lysis buffer
- #7 Flow Through: empty plate for waste collection
- Set program steps:
- Check “Initial Syringe Wash”
- Check “Equilibrate”:
- Volume: 200 µl, Flow Rate: 10 µl/min, Wash Cycles: 1
- Check “Reaction”:
- Volume: 6µl, Wash cycles: 1
- Temperature: 25°C, Duration: 30 min, Reaction Chase: Volume: 100 µl, Flow rate: 10 µl/min
- Check “Cup Wash 1”:
- Volume: 25 µl, Cycles: 2
- Check “Internal Cartridge Wash 1”:
- Volume: 200 µl, Flow rate: 20 µl/min
- Uncheck everything else

5.2 Loading of samples (opt.)

This is optional and depends on the amount of input.
Needs to be repeated as many times as needed.

- Stay with pre-set “On-cartridge reaction” under “**App library”**
- Add the following labware and buffers to the appropriate places:
- #3 Equilibration & Chase Buffer: 250 µl sample
- #5 Wash Buffer 1: 250 µl sample
- #6 Wash Buffer 2: 250 µl sample
- #7 Flow Through: empty plate to collect flow through
- #8 Elution & Syringe Wash buffer: 250 µl sample
- #9 Eluate collection: empty plate to collect flow through
- Set program steps:
- Check “Equilibrate”:
- Volume: 250 µl, Flow Rate: 10 µl/min, Wash Cycles: 0
- Check “Collect Flow Through”
- Check “Internal Cartridge Wash 1”
- Volume: 250 µl, Flow Rate: 10 µl/min, Wash Cycles: 0
- Check “Collect Flow Through”
- Check “Internal Cartridge Wash 2”
- Volume: 250 µl, Flow Rate: 10 µl/min, Wash Cycles: 0

- Check “Collect Flow Through”
- Check “Eluate”
- Volume: 250 µl, Flow Rate: 10 µl/min, Wash Cycles: 0
- Uncheck everything else

5.3 Loading of samples including first wash (>60 min)

- Stay with pre-set “On-cartridge reaction” under “**App library”**
- Add the following labware and buffers to the appropriate places
- #5 Wash Buffer 2: 250 µl samples
- #6 Wash Buffer 2: 250 µl samples
- #7 Flow Through: empty plate to collect flow through
- #8 Elution & Syringe Wash Buffer: 250 µl lysis buffer
- #9 Elutate collection: empty plate to collect waste (BioID lysis buffer)
- Set program steps:
- Check “Internal Cartridge Wash 1”
- Volume: 250 µl, Flow Rate: 10 µl/min, Wash Cycles: 1
- Check “Collect Flow Through”
- Check “Internal Cartridge Wash 2”
- Volume: 250 µl, Flow Rate: 10 µl/min, Wash Cycles: 1
- Check “Collect Flow Through”
- Check “Eluate”
- Volume: 200 µl, Flow Rate: 20 µl/min, Wash Cycles: 1
- Uncheck everything else

5.4 Washing steps (26 min)

- Stay with pre-set “On-cartridge reaction” under “**App library”**
- Add the following labware and buffers to the appropriate places:
- #5 Wash Buffer 1: 320 µl wash buffer
- #6 Wash Buffer 2: 200 µl wash buffer
- Set program steps:
- Check “Cup Wash 1”:
- Volume: 25 µl, Cycles: 2
- Check “Internal Cartridge Wash 1”
- Volume: 250 µl, Flow Rate: 30 µl/min, Wash Cycles: 1
- Check “Cup Wash 2”:
- Volume: 25 µl, Cycles: 2
- Check “Internal Cartridge Wash 2”
- Volume: 125 µl, Flow Rate: 10 µl/min, Wash Cycles: 1
- Uncheck everything else

5.5 LysC digestion and first (mild) elution (>128 min)

- Stay with pre-set “On-cartridge reaction” under “**App library”**
- Add the following buffers to the appropriate places:
  - #4 Reagent: 30 µl digest buffer NB: always make fresh and vortex
  - #5 Wash Buffer 1: 100 µl wash buffer
  - #6 Wash Buffer 2: 100 µl wash buffer
  - #7 Flow Through: empty plate to collect elution
- Set program steps:
  - Check “Reaction”:
    - Volume: 6 µl, Wash Cycles: 1
    - Temperature: 45°C, Duration: 120 min, Reaction Chase: Volume: 0 µl, Flow rate: 0 µl/min
  - Check “Internal Cartridge Wash 1”
    - Volume: 25 µl, Flow Rate: 10 µl/min, Wash Cycles: 1
    - Check “Collect Flow Through”
  - Check “Internal Cartridge Wash 2”
    - Volume: 50 µl, Flow Rate: 10 µl/min, Wash Cycles: 1
    - Check “Collect Flow Through”
  - Uncheck everything else
- Transfer flow through (plate #7) into tubes for further digestion with trypsin.

5.6 Highly acidic elution (>10 min)

- Stay with pre-set “On-cartridge reaction” under “**App library”**
- Add the following labware and buffers to the appropriate places:
  - #3 Equilibration & Chase Buffer: 100 µl elution buffer.

> Note
>
> Always make fresh buffer and vortex

  - #5 Wash Buffer 1: 100 µl elution buffer
  - #6 Wash Buffer 2: 200 µl maintenance buffer
  - #7 Flow through: empty plate to collect elution
  - #8 Stringent Syringe Wash Buffer: 200 µl maintenance buffer
- Set program steps:
  - Check “Equilibrate”:
    - Volume: 15 µl, Flow Rate: 90 µl/min, Wash Cycles: 1
    - Check “Collect Flow Through”
  - Check “Internal Cartridge Wash 1”
    - Volume: 15 µl, Flow Rate: 90 µl/min, Wash Cycles: 1
    - Check “Collect Flow Through”
  - Check “Cup Wash 2”:

- Volume: 25 µl, Cycles: 2
- Check “Internal Cartridge Wash 2”
- Volume: 50 µl, Flow Rate: 10 µl/min, Wash Cycles: 1
- Check “Stringent Syringe Wash”
- Volume: 50 µl, Flow Rate: 5 µl/min, Wash Cycles: 1
- Check “Final Syringe Wash”
- Wash Cycles: 3
- Uncheck everything else
- Transfer flow through (plate #7) into tubes for furtherdigest with trypsin.

5.7 Shut down the robot after the enrichment

- Shut down the Bravo AssayMAP using the pre-set “System shutdown” under “**Utility library”**

## Trypsin digest

6 For first mild elutions:

- Add 0.5 µg of trypsin
- Digest o/n, 37 °C, 500rpm

7 For highly acidic elutions:

- Speed-vac the samples until dry
- Resuspend in 50 µl of 50 mM HEPES
- Check the pH – it needs to be pH 6-8 for Trypsin to work. If not re-buffer with sodium hydroxide
- Add 0.5 µg of trypsin
- Digest o/n, 37 °C, 500rpm

## Clean up

8

- Acidify with 10 % TFA. Check pH, it should be <3, if not add more 10 % TFA
- For both Ambic and ACN elutions, perform **OASIS** clean up according to manufacturer’s instructions
- Store dried peptides until use

## Protocol references

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