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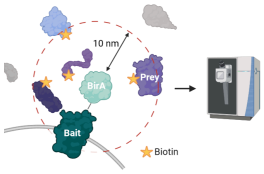
BioID: Identifying Protein-Protein Interactions in Living Cells



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We use this protocol and it's working

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Disclaimer

Abstract

The BioID (proximity-dependent biotin identification) method represents a powerful approach for identifying protein-protein interactions within living cells. This technique utilizes a promiscuous biotin ligase, BirA∗, which is fused to a protein of interest (bait). Upon expression in cells, BirA* biotinylates proximate proteins (prey) in the vicinity of the bait, which can then be isolated using streptavidin affinity purification.
Here, we describe in detail how to process stably expressing HEK cells containing BirA∗-fusion protein and how to capture the biotinylated proteins which are to be subsequently identified through mass spectrometry (DIA).

Materials

Equipment:

- Lasagna plates: Corning 500cm² Square BioAssay Dish (#431110)
- Teat: Pasteur pipette rubber bulb (Carl Roth, #8404.1)
- 50ml Falcon tubes (Corning, #352070)
- 15ml falcon tube (Corning, #352096)
- Serological Pipettes (Greiner, cellstar)
- Liquid nitrogen
- 1.5ml tubes (Eppendorf, #0030 120.086)
- Ice
- Low-binding pipette tips (Sarsted)
- Pierce Spin Columns Snap Cap (Thermo Fisher Scientific, #69725)
- Macro Spin Columns (Harvard Apparatus, #74-4101)
- pH-indicator strips
- Safety cabinet (Thermo, #Safe S2020 1.2)
- Automated cell counter (Biorad, #TC20)
- Trypan blue solution for cell counting (Sigma, #T8154)
- Counting Slides for cells (Biorad, #145-0011)
- Incubator (Thermo, #BBD 6220, CO2 Incubator), settings: 5% CO2, 95%rH, 37°

- Milli-Q water system (Merck, Advantage A10)
- Bioruptor plus (#B01020001, Diagenode)
- Centrifuge 5810R (#5811000015, Eppendorf)
- Heat-block (#SBH130D3 or #SBH200D3, Stuart)
- Thermo Mixer C (#5382000015, Eppendorf)
- OASIS Oasis 96-well plate vacuum manifold (# 186001831, Waters)
- Waters Oasis HLB µElution plates 30 µg (for maximum 100 µg proteins) (#186001828BA, Waters)
- Macro spin columns C-18 (#74-4101, Harvard Apparatus)
- SpeedVac (Concentrator Plus/Vacofuge Plus, #5305000100, Eppendorf)
- Glass vials and glass inserts for LC-MS (#88909355 vials, #93909134 inserts, #88849362 caps, VDS optilab)
- Orbitrap Exploris 480 Mass Spectrometer (Thermo Fisher Scientific)

Reagents:

- Streptavidin coated Sepharose beads (Merck, #GE17-5113-01)
- Sulfo-NHS-Acetate (Thermo Fisher Scientific, #26777)
- *LysC* (Wako, #125-05061, sequencing grade)
- Trypsin (for cell culture) (Thermo Fisher Scientific, #25300-062)
- Trypsin (Mass Spectrometry Grade) (Promega, #V511)
- DMEM high glucose 4.5 g/l (Sigma Aldrich, #D6429)
- FBS (Gibco, #10270-106)
- Biotin (Carl Roth, #3822.1)

- Tetracycline (Sigma Aldrich, #87128)
- Aprotinin (Carl Roth, #A162.3)
- Leupeptin (Carl Roth, #CN33.2)
- Turbonuclease (MoBiTec GmbH, #GE-NUC10700-01)
- Trizma base (Carl Roth, #4855.2)
- Ammonium Bicarbonate (Carl Roth, #T871.2)
- HEPES (Sigma Aldrich, #H3375)
- NaCl (Carl Roth, #3957.1)
- EDTA (Carl Roth, #8043.2)
- EGTA (Carl Roth, #3054.1)
- Triton X-100 (Sigma-Aldrich, #3051.3)
- SDS (Sigma Aldrich, #75746)
- Acetonitrile (Biosolve, #0001204102BS)
- Trifluoroacetic acid (Biosolve, #0020234131BS)
- Methanol (Biosolve, #0013684102BS)
- Formic Acid (Carl Roth, #4724.3)

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

Abbreviations

1 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-aminoethylether)-N,N,N′,N′-Tetraacetic acid
FBS - Fetal Bovine Serum
HEPES - 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
LysC - Native endoproteinase from *Lysobacter enzymogenes*
MetOH – Methanol
PBS - Phosphate-Buffered Saline
RT - Room Temperature
SDS - Sodium Dodecyl Sulfate
TFA - Trifluoroacetic acid
WB - Western Blot
Lasagna - Corning dish for cell culture

Cell Culture

2 Day1: Seed 8e6 cells per lasagna

Note

1 lasagna is normally enough for one replicate of pull-down experiment

3 Day 2: Induce expression of BirA construct by adding Tetracycline 1:1000

Note

Tetracycline stock: [M] 1 mg/mL in Ethanol

4 Day 3: Add [M] 50 micromolar (µM) of Biotin (in water)

5 Day 4: Collect cells

Collection of cells

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

6 Add 4 mL Trypsin per lasagna and incubate 00:07:00 at Room temperature or till cells start to detach 7m

7 Add 2x 20 mL of DMEM with 5% FBS and pipet cells carefully off

8 Spin 00:05:00 , 500 x g, 4°C 5m

9 Remove supernatant and resuspend cells in 10 mL cold PBS

10 Count the cells and prepare tubes with 2e7 containing cells each

11 Spin 00:05:00 , 500 x g, 4°C 5m

12 Remove supernatant and snap freeze samples in liquid nitrogen. Store at -20 °C

Preparation of acetylated beads 2m

13 Equilibrate beads (Streptavidin Sepharose) in PBS by taking 500 µL slurry beads in a new tube

Note

Never vortex or shake the beads too badly to avoid destroying them

13.1 Spin 00:01:00 2000 x g 1m

13.2 Remove supernatant carefully and add 1 mL PBS

13.3 Spin 00:01:00 2000 x g and repeat wash/spin 2x and remove supernatant to have ~400µl left 1m

14 Add freshly made Sulfo-NHS-acetate to a final concentration of 10mM (400µl beads + 360µl PBS + 40 µL [M] 200 millimolar (mM) Sulfo-NHS-Acetate in PBS)

15 Incubate 00:30:00 at RT 30m

16 Add again freshly made (again) Sulfo-NHS-acetate and incubate 00:30:00 at RT 30m

17 Quench with 1:10 of [M] 1 Molarity (M) Tris pH 7.5

18 Wash beads extensively with 1 mL of PBS (at least 2 washes) and spin at 2000 x g for 00:01:00 1m

19 Remove supernatant and resuspend them in PBS (final vol. 500µl)

20 Take out the amount that you need

21 To store the left-over beads, spin them, remove supernatant, wash 2x with 20% ethanol and add 20% ethanol (storage solution) up to the right volume

Pull-down of biotinylated proteins

22 Take one tube per BirA cell line and thaw on ice

Note

You should have 2e7 cells in total per tube

23 Prepare lysis buffer

Note

You need more than just the volume for the samples. You need it later for wash steps, too

	A	B	C	D	E
	Component	Existing conc.	Final Conc.	Vol. needed in µl	Manufacturer
	Tris pH 7.5	500mM	50mM	950	Carl Roth, 4855.2
	NaCl	5M	150mM	285	Carl Roth, 3957.1
	EDTA	500mM	1mM	19	Carl Roth, 8043.2
	EGTA	100mM	1mM	95	Carl Roth, 3054.1
	Triton-X100		1%	95	Carl Roth, 3051.3
	Aprotinin	10mg/ml Stock	1/1000	9.5	Carl Roth A162.3
	Leupeptin	5ml/ml Stock	1/1000	9.5	Carl Roth, CN33.2
	Turbonuclease		250U	1	MoBiTec GmbH, GE-NUC10700-01
	SDS	20%	0.10%	47.5	
	Water			7988.5	

In the table above are the amounts needed for one sample

24 Resuspend the pellet in 4.75 mL of Lysis buffer in a 15ml falcon tube

25 Lyse the cells by rotating the falcon tube for 01:00:00, 15 rpm, 4°C 1h

26 Split each sample into 2×2ml tubes

27 Sonicate the sample 10× 30sec on/off with the Bioruptor at 4 °C

Note

No visible aggregates should be there, otherwise sonicate more

28 Spin the samples 00:30:00, 17000 x g, 4°C → keep supernatent for further analysis 30m

Equilibrate the (already prepared) beads in the meantime

29 Rebuffer the beads in PBS (wash 3x with PBS, spin at 2000 x g for 00:05:00, remove supernatant) 5m

30 Add 80 µL of slurry beads to 1 mL Lysis buffer

31 Incubate them for 00:30:00 15 rpm, 4°C 30m

32 Spin 00:05:00, 200 x g 4 °C 5m

33 Remove as much of the Lysis buffer as possible

Continue with pull-down of biotinylated proteins

3h 5m

34 Transfer the supernatants per sample in a fresh 15ml falcon tube

35 Add equilibrated beads, use some additional supernatant of the sample to transfer all the beads

36 Incubate 03:00:00 15 rpm, 4°C 3h

37 Spin 00:05:00 , 2000 x g 4 °C 5m

38 Remove 4.5ml of supernatant → keep 50µl of each sample for WB (flow-through)

39 Transfer the rest to one of the “empty” columns (Pierce Spin Columns Snap Cap)

40 Rinse falcon tube with 500 µL of Lysis buffer to transfer all the beads

41 Wash beads with 800 µL of Lysis buffer

42 Wash beads 5x with 600 µL [M] 50 millimolar (mM) AmBic, pH 8.3

> Note
>
> use freshly prepared AmBic

43 Close column with a plug on the bottom

44 Transfer the beads to a 2ml tube and spin 2000 x g , 00:05:00 , 4 °C 5m

45 Remove supernatant to have 200µl left (~700-720µl)

46 Add 1 µL of LysC ([M] 1 µg/µL)

47 Digest 16:00:00 , 37 °C , 500 rpm 16h

48 Spin 2000 x g , 00:05:00 , Room temperature 5m

49 Transfer everything to an “empty” column (Pierce Spin Columns Snap Cap)

50 Elute digested peptides

51 Add 2x 150 µL of [M] 50 millimolar (mM) AmBic and pipet 5x up and down

52 Collect all the elutions of digested peptides together in a low-binding tube

53 Add 2x 150 µL of 80% ACN + 20% TFA and pipet 5x up and down (fast)

54 Collect all the elutions of digested peptides together in a low-binding tube

For AmBic elutions 3h

55 Add 0.5 µL of Trypsin ([M] 1 µg/µL)

56 Digest 03:00:00 , 500 rpm, 37°C 3h

57 Speed-vac the samples

58 Resuspend in 200 µL OASIS Buffer A, sonicate 00:01:30 1m 30s

59 Check pH, it should be <3, if not acidify with 10% TFA

For ACN elutions 3h

60 Speed-vac the samples to near dryness (~50µl)

61 Add 50 µL of [M] 200 millimolar (mM) HEPES pH 8.0

> Note
>
> Check the pH – it needs to be pH 6-8 for Trypsin to work. If not re-buffer with sodium hydroxide (for example add 3µl of 1N NaOH).

62 Add 0.5 µL of Trypsin ([M] 1 µg/µL)

63 Digest 03:00:00 , 500 rpm, 37°C 3h

64 Acidify with 10 µL of 10% TFA (check the pH afterwards).

Clean up, for both AmiBic and ACN elutions 3h

65 Perform MACRO-SPIN clean-up (capacity 30-300µg) (spin always 1000 x g)

66 Equilibrate column with 500 µL of 100% MetOH. Spin 00:01:00 . 1m

67 Wash 2x 300 µL 5% ACN, 0.1% Formic Acid. Spin 00:01:00 . 1m

68 Load sample 2x(50-450µl). Spin 00:01:00 . 1m

69 Wash 4x 300 µL 5% ACN, 0.1% Formic Acid. Spin 00:01:00 . 1m

70 Elute 2x 250 µL 50% ACN, 0.1% Formic Acid. Spin 00:01:00 . 1m

71 SpeedVac to dryness.

72 Resuspend in 15 µL of MS Buffer A
For AmBic elutions take 10 µL of the sample into a MS vial and inject 5 µL for DIA analysis.
For ACN elutions take 10 µL of the sample into a MS vial and inject 3 µL for DIA analysis.

Note

Please note that the ACN elutions will still contain some background peptides from Streptavidin, thus run them AFTER the AmBic elutions and check for carry over after your runs!!

73 Store the remaining @ -20°C.

Protocol references

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