

Characterisation data for all proteins detailed in text:

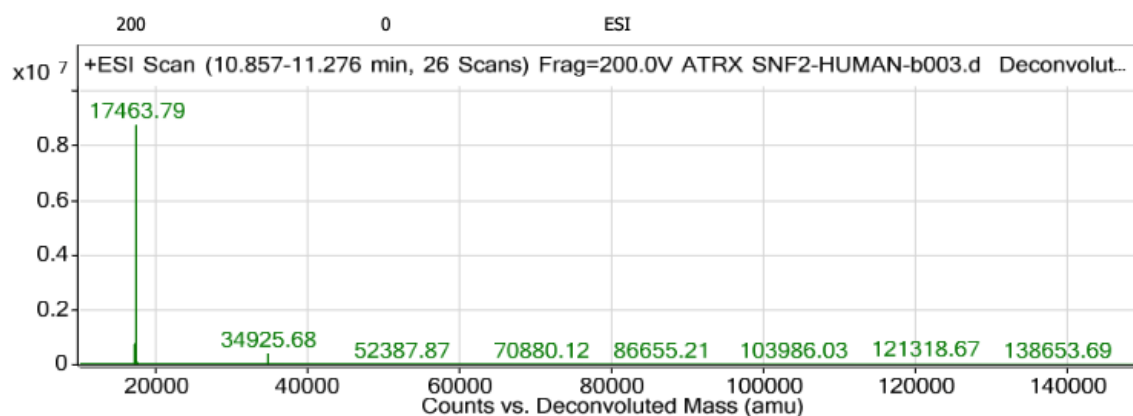
His-tagged DID_{ATRX} (1190-1325)

MHHHHHHSSGVDLGTEENLYFQSMRTSTKRKQADITSSSSSDIEDDDQNSIGEGSSDEQKIKPVTENLVLS
HTGFCQSSGDEALSKSVPVTVDDDDDDNDPENRIAKKMLLEEIKANLSSDEDGSSDDEPEEGKKRTGKQ
NEENPGDEEAKNQVNSESD

Coomassie-stained SDS-PAGE gel:



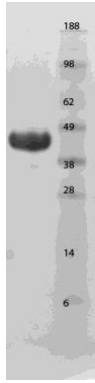
Mass-spec analysis: Expected 17.462 kDa. MS result 17.464 kDa (-2)



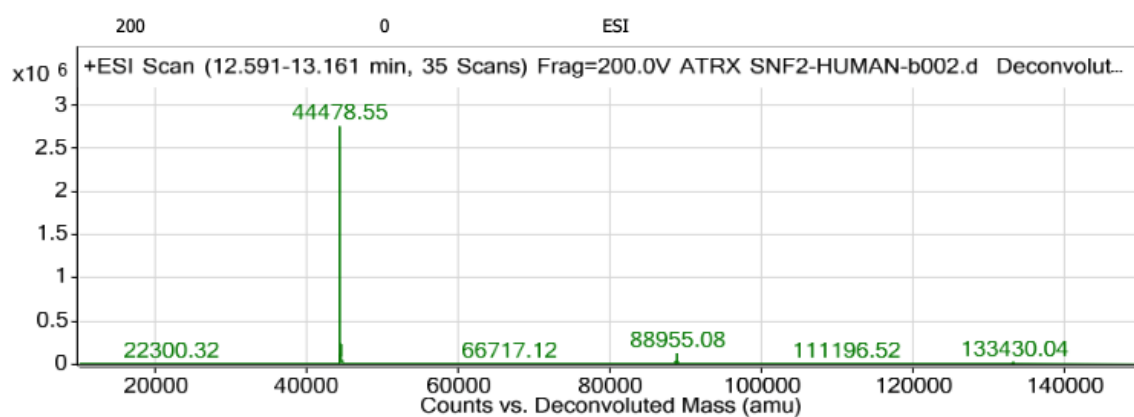
GST-His-tagged DID_{ATRX}

MSDKIIHSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQ
SMAIIRYIADKHNMLGGCPKERAIEISMLEGAVLDIRYGVSRAYSKDFETLKVDLFLSKLPEMLKMFEDRLCH
KTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGQWQAT
FGGGDHPPKLEVLFGQHMHHHHHHSSGVDLGTEENLYFQSMRTSTKRKQADITSSSSSDIEDDDQNSIGE
GSSDEQKIKPVTENLVLSSTHTGFCQSSGDEALSKSVPVTVDDDDDDNDPENRIAKKMLLEEIKANLSSDED
GSSDDEPEEGKKRTGKQNEENPGDEEAKNQVNSESD

Coomassie-stained SDS-PAGE gel:



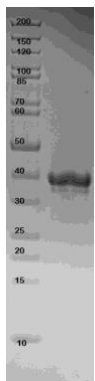
Mass-spec analysis: Expected 44.561 kDa. MS result 44.479 kDa (-82)



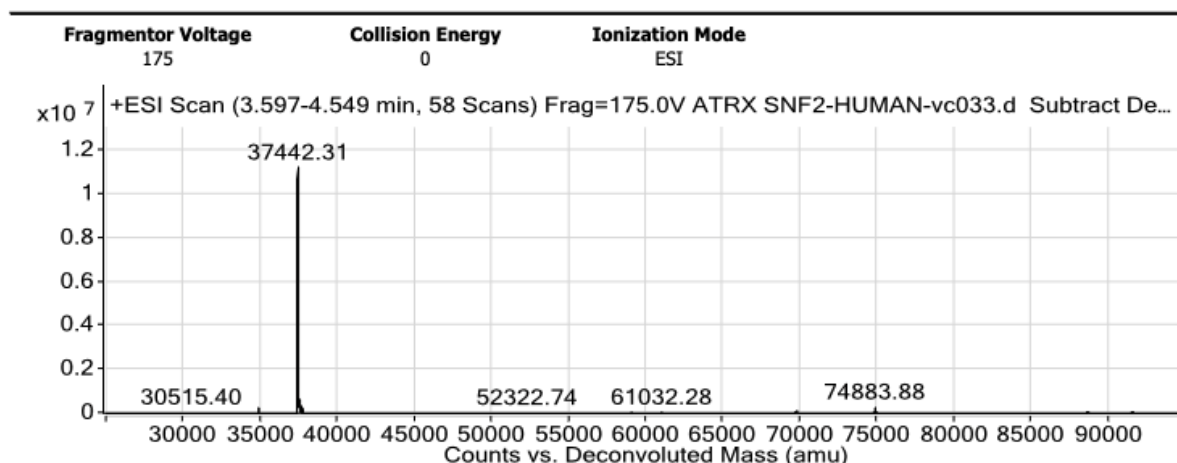
GST-His-tagged ATRX 1194-1266

MSDKIIHSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQ
 SMAIRYIADKHNMLGGCPKERAISMLEGAVLDIRYGVSRAYSKDFETLKVDFLSKLPEMLKMFEDRLCH
 KTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGWWQAT
 FGGGDHPPKLEVLFFQGHMHHHHHHSSGVDLG TENLYFQSMKRKQADITSSSSSDIEDDDQNSIGEGSSD
 EQKIKPVTENLVLSHTGFCQSSGDEALSKSVPVTVDDDDDDND

Coomassie-stained SDS-PAGE gel:



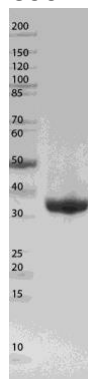
Mass-spec analysis: Expected MW 37.573 kDa. MS result 37.573 (-131)



GST-His-tagged ATRX 1267-1325

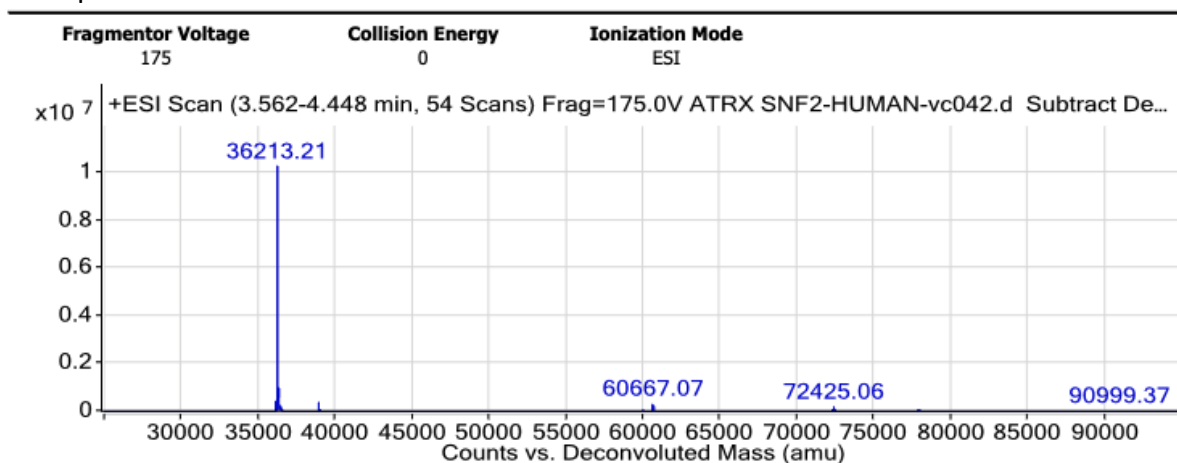
MSDKIIHSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQ
 SMAIIRYIADKHNMLGGCPKERAISMLEGAVLDIRYGVSRAYS KDFETLKVDFLSKLPEMLKMFEDRLCH
 KTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSSKYIAWPLQGWQAT
 FGGGDHPPKLEVL FQGHMHHHHHHSSGVD LGTENLYFQSMPENRIAKKMLLEEIKANLSSDEDGSSDD
 EPEGKKRTGKQNEENPGDEEAKNQVNSESD

Coomassie-stained SDS-PAGE gel:



Mass-spec analysis: Expected MW 36.343 kDa. MS result 36.213 (-130)

MS report:



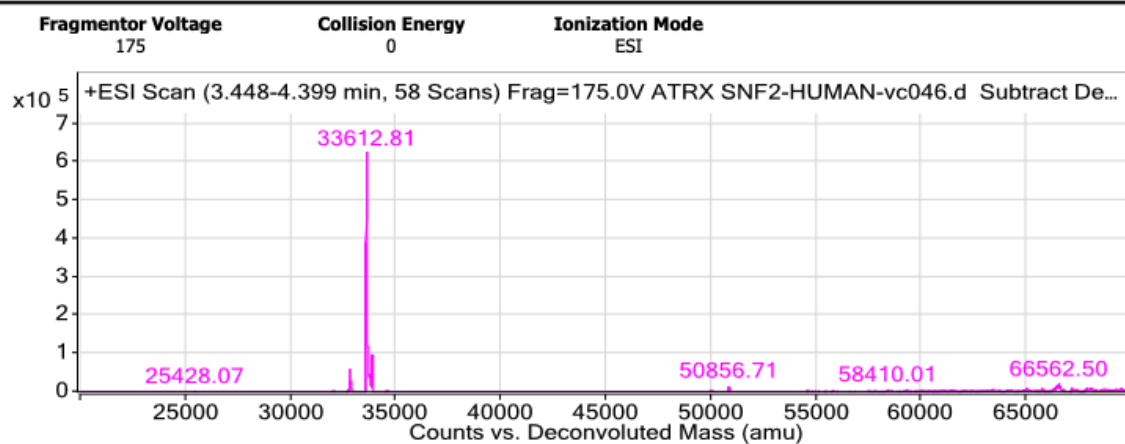
GST-His-tagged ATRX 1255-1289

MSDKIIHSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQ
SMAIIRYIADKHNMLGGCPKERAISMLEGAVLDIRYGVSRAYS KDFETLKVDFLSKLPEMLKMFEDRLCH
KTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQAT
FGGGDHPPKLEVL FQGHHHHHHSSGVDLG TENLYFQSMPTVDDDDDDNDPENRIAKKMLLEEIK
ANLSSDED

Coomassie-stained SDS-PAGE gel:



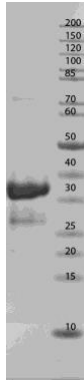
Mass-spec analysis: Expected MW 33.743 kDa. MS result 33.613 (-130)



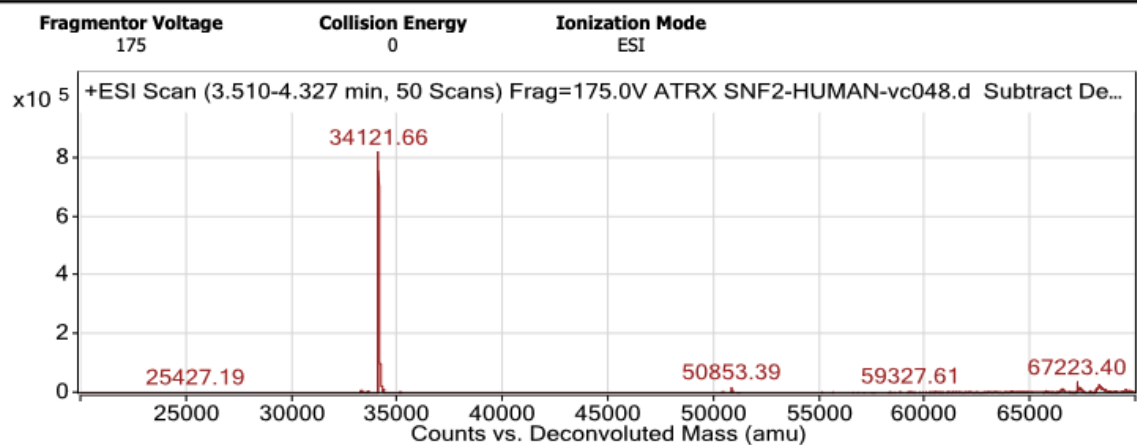
GST-His-tagged ATRX 1285-1325

MSDKIIHSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQ
SMAIIRYIADKHNMLGGCPKERAISMLEGAVLDIRYGVSRAYS KDFETLKVDFLSKLPEMLKMFEDRLCH
KTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQAT
FGGGDHPPKLEVL FQGHHHHHHSSGVDLG TENLYFQSMSSDEDGSSDDEPEEGKKRTGKQNEENP
GDEEAKNQNSESD

Coomassie-stained SDS-PAGE gel:



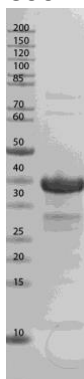
Mass-spec analysis: Expected MW 34.251 kDa. MS result 34.122 (-129)



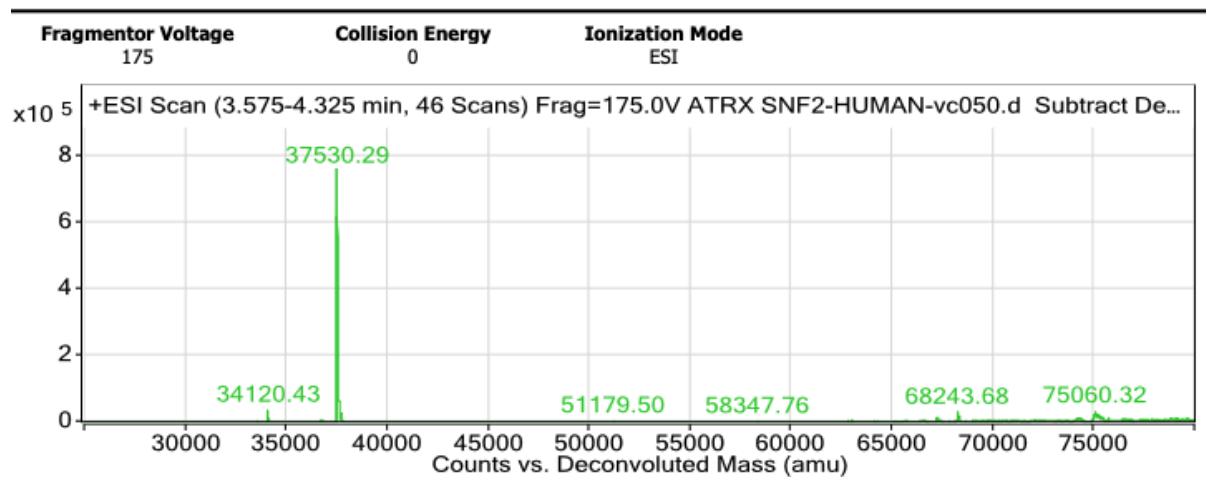
GST-His-tagged ATRX 1255-1325

MSDKIIHSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQ
 SMAIIRYIADKHNMLGGCPKERAIEISMLEGAVLDIRYGVSRAYS KDFETLKVDFLSKLPEMLKMFEDRLCH
 KTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKYLKSSKYIAWPLQGWQAT
 FGGGDHPPKLEVL FQGHMHHHHHSSGVDLG TENLYFQSMPVTVD DDDDDNDPENRIAKKMLLEEIK
 ANLSSDEDGSSDDEPEEGKKRTGKQNEENPGDEEAKNQVNSESD

Coomassie-stained SDS-PAGE gel:



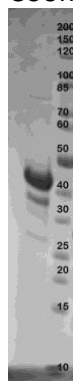
Mass-spec analysis: Expected MW 37.660 kDa. MS result 37.530 (-130)



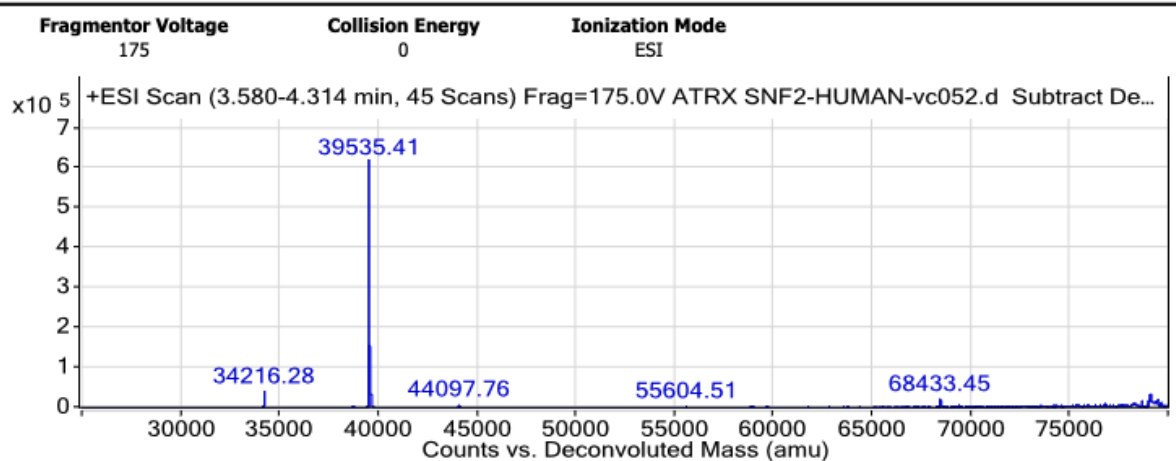
GST-His-tagged ATRX 1194-1284

MSDKIIHSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQ
 SMAIIRYIADKHNMLGGCPKERAISMLEGAVLDIRYGVSRAYSKEFETLKVDFLSKLPEMLKMFEDRLCH
 KTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIQIDKYLKSSKYIAWPLQGWQAT
 FGGGDHPPKLEVLFFQGHMHHHHHHSSGVDLG TENLYFQSMKRKQADITSSSSSDIEDDDQNSIGEGSSD
 EQKIKPVTENLVLSHTGFCQSSGDEALSKSVPVTVD DDDDDNDPENRIAKKMLLEEIKANL

Coomassie-stained SDS-PAGE gel:



Mass-spec analysis: Expected MW 39.665 kDa. MS result 39.535 (-130)



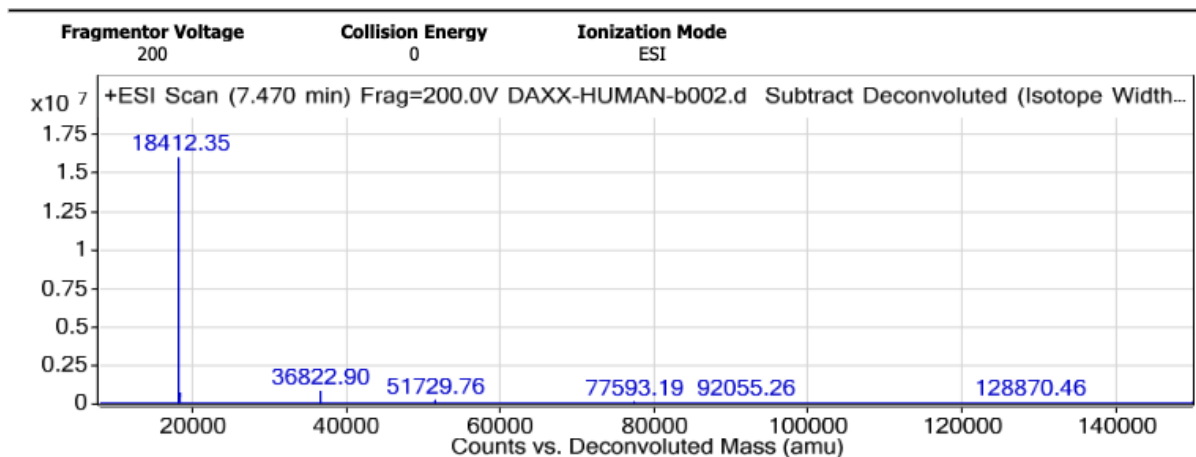
NSIM-DHB_{DAXX} (1-144)

MHHHHHHSSGVDLG TENLYFQSM MATANSIIVLDDDDDEDEAAAQPGPSHP
LPNAASPGAEAPSSSEPHGARGSSSSGGKKCYKLENEKLFEEFLELCKMQ
TADHPEVVPFLYNRQQRAHSLFLASAEFCNILSRVLSRARSRAKLYVYI
NELCTVLKAHS AKKKLN

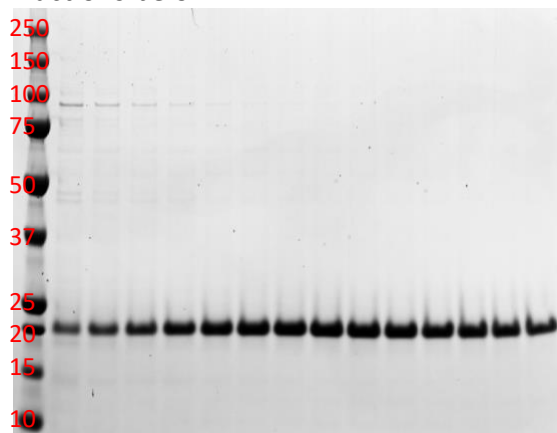
Coomassie-stained SDS-PAGE gel:



Mass-spec analysis: Expected MW 18.412 kDa. MS result 18.412



His-tag was subsequently cleaved, passed over NiNTA resin and the unbound fraction purified by size exclusion chromatography. Coomassie-stained SDS-PAGE gel of purified fractions below:



¹⁵N labelled NSIM-DHB_{DAXX} (1-144)

MHHHHHHSSGVDLG TENLYFQSM MATANSIIVLDDDDDEDEAAAQPGPSHP
 LPNAASPGAEAPSSSEPHGARGSSSSGGKKCYKLENEKLFEEFLELCKMQ
 TADHPEVVPFLYNRQQRAHSLFLASAEFCNILSRVLSRARSRP AKLYVYI
 NELCTVLKAHSAKKKLN

Coomassie-stained SDS-PAGE gel of final purified protein:

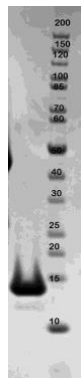


Expected MW after tev cleavage 16.054 kDa.

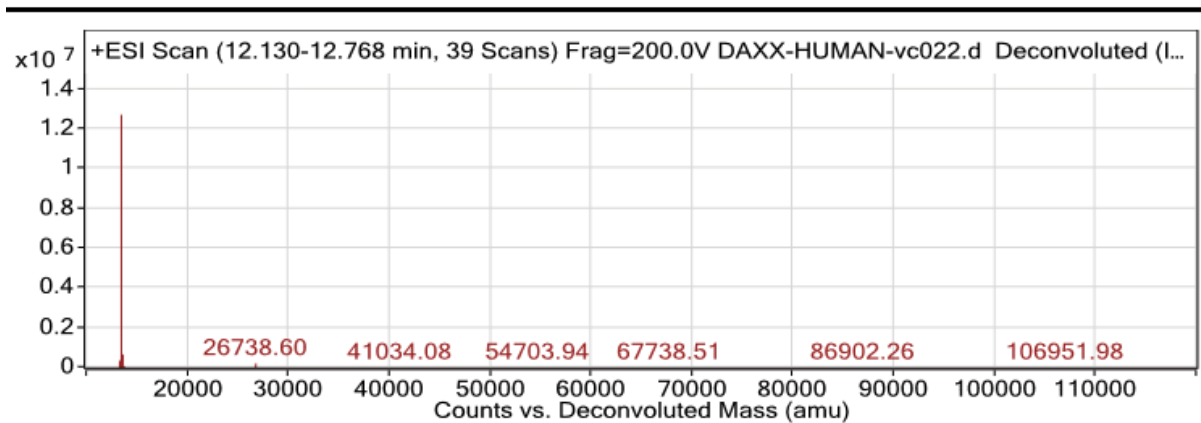
DHB_{DAXX} (55-144)

MHHHHHHSSGVDLG TENLYFQSMGKKCYKLENEKLFEEFLELCKMQTADH
 PEVVPFLYNRQQRAHSLFLASAEFCNILSRVLSRARSRP AKLYVYINELC
 TVLKAHSAKKKLN

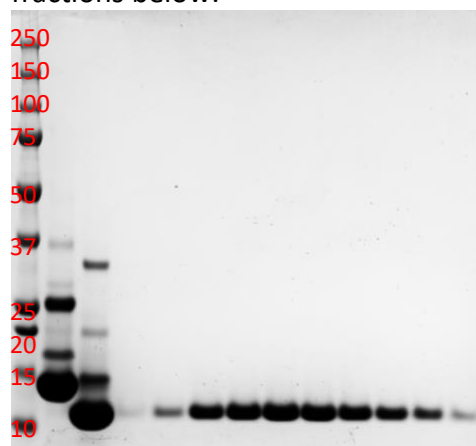
Coomassie-stained SDS-PAGE gel:



Mass-spec analysis: Expected MW 13.202 kDa. MS result 13.203



His-tag was subsequently cleaved, passed over NiNTA resin and the unbound fraction purified by size exclusion chromatography. Coomassie-stained SDS-PAGE gel of purified fractions below:

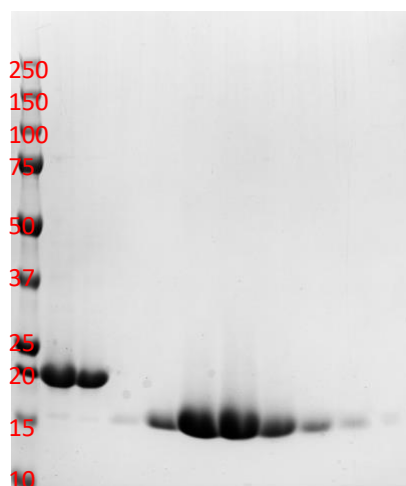


Lanes: 1. Precision plus markers. 2. Before tev cleavage. 3. After tev cleavage 4-13. Fractions after size exclusion chromatography.

SUMO-1 (1-97)

MGSSHHHHHHSSGENLYFQGHMSDQEAKPSTEDLGDKKEGEYIKLVIGQ
DSSEIHFKVKMTTHLKKLKESYCQRQGVPMNSLRFLFEGQRIADNHTPKE
LGMEEDVIEVYQEQTGG

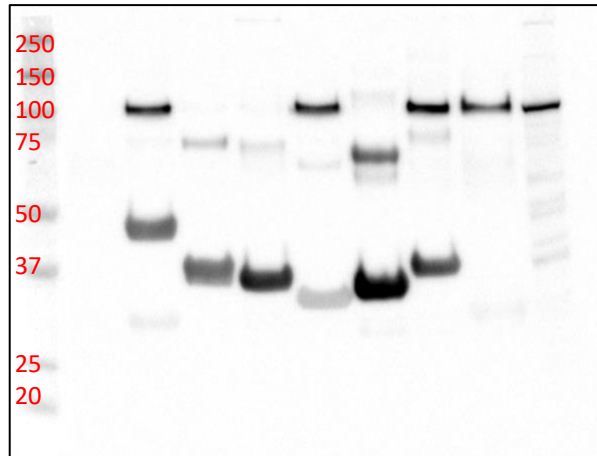
Coomassie-stained SDS-PAGE gel of final purification step (after tev cleavage):



1. Precision plus markers
2. ^{15}N -labelled NSIM-DHB_{DAXX}
3. Unlabelled NSIM-DHB_{DAXX}
- 4-11 Fractions of SUMO-1 eluted from size exclusion column.

Expected MW (after tev cleavage) 11.270 kDa.

Full western blot (Fig. 1)



1. Markers
2. Beads
3. DID_{ATR}X (44.5 kDa)
4. ATRX 1194-1266 (37.6 kDa)
5. ATRX 1267-1325 (36.3 kDa)
6. ATRX 1255-1289 (33.7 kDa)
7. ATRX 1285-1325 (34.3 kDa)
8. ATRX 1255-1325 (37.7 kDa)
9. ATRX 1194-1284 (39.7 kDa)
10. 2.5 % input

Cross-reactivity of DAXX antibody with GST-tagged proteins observed, presumably due to very high amounts of protein on membrane. DAXX runs large (MW = 81.4 kDa)