Supplemental Information. Detailed description of KingFisher bead handling method.

[PROTOCOL PROPERTIES]

Name = fhcrc_pcr_25ul_4wash
Protocol template version = 2.6.0
Instrument type = KingFisher 96
Creator = FHCRC
Plate layouts = Plate_1_beads wash, Plate_2_plasma_pep_Ab, Plate_3_wash_1, Plate_4_wash_2, Plate_5_wash_3, Plate_6_wash_4, Plate_7_elution, plate_8_tip

[PLATE LAYOUTS]

Plate_1_beads wash

Plate type = KingFisher 96 plate
Plate change message = Plate_1_beads wash

A:
- volume = 200, name = beads, pbs 0.03% chapsPlate_2_plasma_pep_Ab

Plate type = KingFisher 96 plate
Plate change message = Plate2_PlasmaPepAb

A:
- volume = 100, name = plasma/peptide/Ab/pbs 0.03%chapsPlate_3_wash_1

Plate type = KingFisher 96 plate
Plate change message = Plate3_Wash1

A:
- volume = 200, name = wash buffer (pbs 0.03% CHAPS pH 7.50)Plate_4_wash_2

Plate type = KingFisher 96 plate
Plate change message = Plate4_Wash2

A:
- volume = 200, name = wash buffer (pbs 0.03% CHAPS pH 7.5)Plate_5_wash_3

Plate type = KingFisher 96 plate
Plate change message = Plate5_Wash 3

A:
- volume = 200, name = pbs 0.03%chapsPlate_6_wash_4

Plate type = KingFisher 96 plate
Plate change message = Plate6_wash_4

A:
- volume = 200, name = pbs 1/10 0.03%chapsPlate_7_elution

Plate type = KingFisher 96 plate
Plate change message = Plate_7_elution

A:

- volume = 50, name = 5% acetic acidplate_8_tip

Plate type = KingFisher 96 plate
Plate change message =

A:
- EMPTY

[STEPS]

MIX
Step parameters
Name = Mix_Beads
Plate = Plate_1_beads wash

Beginning of step:
Release = Yes, time = 10s, speed = Slow

Mix parameters:
Mix time = 5min 0s, speed = Bottom slow

End of step:
Collect beads = Yes, count = 5
BIND
Step parameters
Name = Bind_Bead Capture
Plate = Plate_2_plasma_pep_Ab

Beginning of step:
Release = Yes, time = 10s, speed = Slow

Bind parameters:
Bind time = 2h 0min 0s, speed = Bottom very slow

End of step:
Collect beads = Yes, count = 5
WASH
Step parameters
Name = Wash_1 wash buffer
Plate = Plate_3_wash_1

Beginning of step:
Release = Yes, time = 10s, speed = Slow

Wash parameters:
Wash time = 1min 0s, speed = Slow

End of step:
Collect beads = Yes, count = 5
WASH
Step parameters
Name = Wash_2 wash buffer
Plate = Plate_4_wash_2

Beginning of step:

Release = Yes, time = 10s, speed = Slow

Wash parameters:
Wash time = 1min 0s, speed = Slow

End of step:
Collect beads = Yes, count = 5
WASH
Step parameters
Name = Wash_3 wash buffer
Plate = Plate_5_wash_3

Beginning of step:
Release = Yes, time = 10s, speed = Slow

Wash parameters:
Wash time = 1min 0s, speed = Slow

End of step:
Collect beads = Yes, count = 5
WASH
Step parameters
Name = Wash_4 wash buffer
Plate = Plate_6_wash_4

Beginning of step:
Release = Yes, time = 10s, speed = Slow

Wash parameters:
Wash time = 1min 0s, speed = Slow

End of step:
Collect beads = Yes, count = 5
ELUTION
Step parameters
Name = Elution
Plate = Plate_7_elution

Beginning of step:
Release = Yes, time = 10s, speed = Slow

Elution parameters:
Elution time = 5min 0s, speed = Slow
Heating = No

Remove beads:
Remove beads = Yes, collect count = 10, disposal plate = Plate_1_beads wash