Protein Labeling and Tethering Kit (ybbR)

1. Protein Labeling with DNA Handles

1.1 Conjugation of CoA–modified oligo to protein (Step 1)

1. Bring the protein of interest solution to a final volume of 15 µl and final concentration of 50 µM with the buffer of choice.
2. Prepare the following reaction by first mixing CoA–modified oligo with 10x Sfp reaction buffer and TCEP. Then add the protein of interest and Sfp enzyme.
3. Incubate at room temperature for 2 hours.

Components	Volume
CoA–modified oligo (100 µM)	20 µl
10x Sfp reaction buffer	4 µl
TCEP (100 mM)	1 µl
Protein of interest (50 µM)	15 µl
Sfp enzyme	5 µl

1.2 Purification of protein-oligo chimera (Step 2)

1. For 6xHis– and GST– tagged protein, His SpinTrap™ (GE28-4013-53) and GST SpinTrap™ (GE28-9523-59) are recommended. For a detailed purification protocol, please refer to “6xHis-AdK-oligo purification” protocol in the Product Resources webpage.
2. Measure the concentration of the purified protein–oligo by reading the absorbance at 260 nm.
3. Ensure the protein–oligo concentration is at least 20 ng/µl for optimal results in the next step.
4. Make aliquots of the protein–oligo chimera, snap-freeze them, and store at –80 °C.

1.3 Annealing to DNA handles (Step 3)

Use 4 µl for the 0.5 and 1.5 kb handles, and 6 µl for the 5 kb handles.

1. Dilute the protein–oligo chimera to a final concentration of 20 ng/µl using a buffer containing Tris–HCl 20–50 mM and 50–150 mM NaCl (pH 7.5).
2. Add 1 µl of the protein–oligo chimera to the DNA handles.
3. Incubate at room temperature for 10 min to allow annealing. Then, keep the sample on ice.

Components	Volume
Protein–oligo chimera (20 ng/µl)	1 µl
DNA handles (0.5, 1.5 or 5 kb)	4 µl or 6 µl

2. Protein Tethering in the C-Trap

2.1 Microfluidics Setup

Channel 1:

- For **0.5 and 1.5 kb handles**, dilute the protein–DNA handle complex 1:15.
- For **5 kb handles**, no dilution is required.

1. Add 5 µl of the (diluted or undiluted) protein–DNA handle complex to 5 µl anti-digoxigenin beads. Mix gently by pipetting 3–5 times, avoiding bubbles.
2. Incubate at room temperature for 5 minutes.
3. Dilute the bead–protein complex in 500 µl of buffer and load into the syringe of channel 1.

Channel 2: Load 500 µl buffer (use oxygen scavengers if using 0.5 kb or 1.5 kb handles).

Channel 3: Add 1 µl of streptavidin beads to 1 ml buffer, mix gently and load in the syringe.

2.2 Positioning Trap 1 and Trap 2

1. Catch one bead in each trap; bead identity is not important at this stage.
2. Activate “Show Trap 1 overlay” and ensure that the bead is positioned in the Trap 1 overlay by Shift + Left–clicking on the bead.
3. Move Trap 1 to the top of the red rectangle and Trap 2 to the bottom, then save the position of Trap 1 as “catching”.
4. Catch an anti-digoxigenin bead in Trap 2 (channel 1), then move Trap 1 to channel 3 and catch a streptavidin bead. The pre-set catching position keeps Trap 2 in channel 2 while trapping with Trap 1 in channel 3.
5. Align the beads horizontally with Trap 1 at a 5 µm distance. Save this position as “tethering”.
6. Perform trap calibration according to the different sizes of the two beads.

2.3 DNA Tethering

1. Set the WLC model to the contour length of two DNA handles (1.034, 3.012, or 10.078 kb for 0.5, 1.5, or 5 kb)
2. **For 5 kb:** use default tracking and templates; apply 0.3–0.5 bar flow and move Trap 1 to 50–60% of contour length (~1.5–1.7 µm).
3. Increase distance to test for tether formation; if no tether is formed, reduce tethering distance (<1.5 µm) and/or wait a few seconds before retrying.
4. **For 0.5 and 1.5 kb:** enable piezotracking; flow is not required
5. Record an FD baseline by moving Trap 1 from 5 µm to 60% of contour length to correct for bead interaction at short distances of tethering (<0.5 µm).
6. Proceed with tethering by positioning Trap 1 at 50–60% of the contour length, as done for 5 kb handles.