Biotinylated double-stranded DNA (48,502 bp)

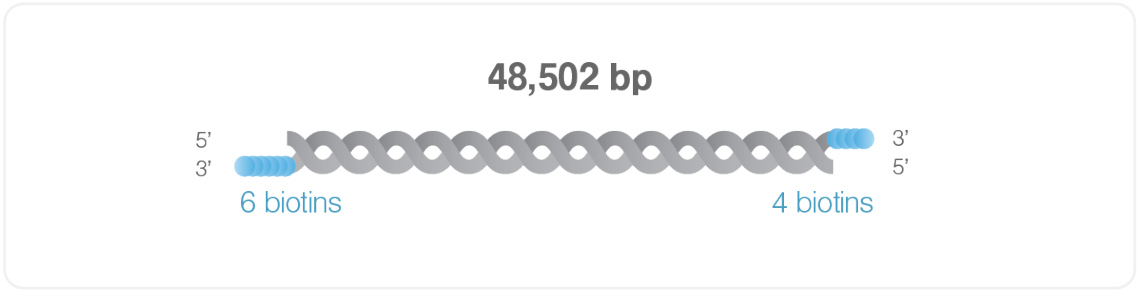
DNA–Protein Interations Protocol

2021

LUMICKS

Biotinylated double-stranded DNA (48,502 bp)

The length of the nick-free biotinylated double-stranded λ-DNA (48,502 bp) makes it ideal for assessing different types of DNA binding molecules.



Materials supplied in the kit

All the components of the kit are stable for 6 months when stored correctly. The λ-DNA can be used with streptavidin coated beads. We recommend using the ⌀ 4.0–4.9 µm beads, available in our store.

Components	Units	Volume	Storage temperature
Biotinylated double-stranded DNA (48,502 bp)	1	20 µl	+4 ˚C

Experimental set-up for the microfluidics system with laminar flow cell

Prepare the following conditions for the different channels of the microfluidics system and load them in their corresponding syringes.

Channel 1

- Dilute running buffer 10 times (creating PBS with 5 mM sodium azide and 0.5 mM EDTA) and use 1 ml for channel 1.
- Add 10 µl of streptavidin-coated polystyrene beads (4.0–4.9 µm, 0.5% (w/v)).

Channel 2

- Dilute running buffer 10 times (creating PBS with 5 mM sodium azide and 0.5 mM EDTA) and use 1 ml for channel 2.
- Add 1 µl biotinylated double-stranded DNA (48,502 bp) (20 ng/µl).

Channel 3

- Dilute running buffer 10 times (creating PBS with 5 mM sodium azide and 0.5 mM EDTA) and use 1 ml for channel 3.

Catching beads and DNA tethering

- Apply flow to channels 1, 2, and 3 with a pressure of 0.3–0.4 bar.
- Catch a single streptavidin-coated bead in each of the traps in channel 1 and while being aligned on the y-axis move these to channel 2.
- Keep the flow of channels 1-3 open and oscillate the trap on the right back and forth and determine if there is a tether formed by observing the force response. You will be able to discriminate between single or multiple tethers by looking how the real-time force-distance data (displayed on the F,d Bluelake tab) matches the worm-like chain model for lambda DNA printed on the same tab. The presence of multiple tethers between the beads will appear as a force rise at a shorter distance than the distance predicted by the worm-like chain model. In addition, the DNA overstretching plateau will appear at higher forces proportionally to the number of tethers between the beads (i.e. around 65 pN for one molecule, 120 pN for two, and so forth).
- Aim for forming a single tether between the beads. In case of multiple tethers, they can be broken in channel 3 by increasing the force until a single tether remains. In case multiple tethers are regularly being formed the DNA concentration can be lowered.
- Move the single DNA tether to channel 3 and turn off the flow in all channels. Here, a force distance curve can be recorded to validate that there is only a single DNA tether.

info@lumicks.com
www.lumicks.com

Or find us on:

LUMICKS HQ

Paalbergweg 3
1105 AG Amsterdam, The Netherlands
+31 (0)20 220 0817

LUMICKS USA

800 South Street, Suite 100,
Waltham, MA, 02453, USA
+1 857 209 4097

LUMICKS Asia

Room 545, Block A, Langentbldg Center
No.20 East Middle 3rd Ring Road
Chaoyang District, Beijing, 100022 China
+86 (0) 10 5878 3028

LUMICKS