

BG4 Indirect Immunofluorescence Protocol

Hänsel-Hertsch R.; Beraldi D.; Lensing S. V.; Marsico G.; Zyner K.; Parry A.; Di Antonio M.; Pike J.; Kimura H.; Narita M.; Tannahill D.; Balasubramanian S. G-quadruplex structures mark human regulatory chromatin. Nat. Genet. 2016, 48, 1267–1272.1038/ng.3662

- 1) Seed cell line of choice on sterile coverslips the night before harvest so that they will be 50% to 80% confluent. Use 24mm (circular coverslips) pre-coated with 0.1mg/mL poly-D-lysine (Sigma, cat. no. P7405) in the bottom of a 12-well plate.
- 2) Fix using 1mL of 4% paraformaldehyde in 1 x PBS for 10 min at RT.
- 3) Rinse carefully with 2mL PBS.
- 4) Permeabilise with 1 x PBS containing 0.1% Triton-X for 10 min at RT.
- 5) Rinse x2 with PBS .
- 6) *Optional: RNase A treat coverslips with 20 μ L 10mg/mL RNaseA + 800 μ L PBS: (50 μ L per coverslip). Incubate for 1 h at 37 degrees. This should reduce any cytoplasmic foci to background and leave the majority of nuclear foci untouched.*
- 7) *Optional: DNase treat cells using 60U Turbo DNase (ThermoFisher cat. no. AM2238) in 1 x Turbo DNase buffer (50 μ L per coverslip) and incubate at 37 degrees for 1 h. This should remove most nuclear foci and not touch cytoplasmic foci.*
- 8) Block coverslips for 1h with 0.5% goat serum in PBST (PBS + 0.1% Tween20) at 37 degrees.
- 9) Incubate coverslips with the BG4 antibody diluted (1:100 in 0.5% goat serum + PBST (50 μ L per coverslip) and incubate at 37 degrees for 1 h. We suggest that, for optimal results, the antibody is titred; typically the stock of BG4 antibody that we make is ~6-8 μ M.
- 10) Wash three times with PBST for 5 min each.
- 11) Incubate with 1:800 Rabbit anti-FLAG (cat. no. 2368, Cell Signaling Technology) in PBST + 0.5% goat serum for 1 h at 37 degrees (50 μ L per coverslip).
- 12) Wash three times with PBST for 5 mins each.
- 13) Incubate with secondary antibody diluted in in PBST + 0.5% goat serum for 1 h at RT e.g. Rabbit Alexa Fluor 594 1:500- 1:1000 (ThermoFisher cat. no. A11037).
- 14) Wash three times with PBST for 5 min each (second wash containing DAPI).
- 15) Rinse 3 times with RO water.
- 16) Air dry.
- 17) Mount coverslips on Superfrost Plus slides (cat. no. 4951PLUS) with ThermoFisher ProLong Diamond anti-fade mounting medium (cat. no. P36970).

Notes:

- a) We only recommend the single chain phage display version. The pSANG10-3F-BG4 expression plasmid is available from Addgene (Plasmid #55756) and the antibody is available from Merck in limited quantities (cat. no MABE917). We have not tested the BG4 format available from Absolute Antibody.
- b) Methanol/acetic acid may be a better fixative for some cell types/species.
- c) We find that the use of goat serum rather than milk allows detection of weaker signals.