Frequently Asked Questions

- **What does the product look like?**
  - Appearance:
    - The products are supplied as a dried film of blue material adhering to the inside of the conical bottom of a ‘champagne flute’ brown glass vial
      - The product should be visible as a dark smear through the side of the vial

- **The fluorophore is red, but the solution is blue - have I made it up properly?**
  - Reconstitution:
    - If dissolved in DMSO at 10mM according to the instructions in the datasheet, the product will appear to be a dark blue solution – this is correct
      - For sub-aliquoting in slightly larger volumes, the product can also be reconstituted at 1mM by adding 10x the volume of DMSO stated
    - The product can be further diluted in DMSO to eg 100µM and will retain its blue colour
      - Final dilution to ~ 100nM is in buffer (+ 0.1% DMSO)

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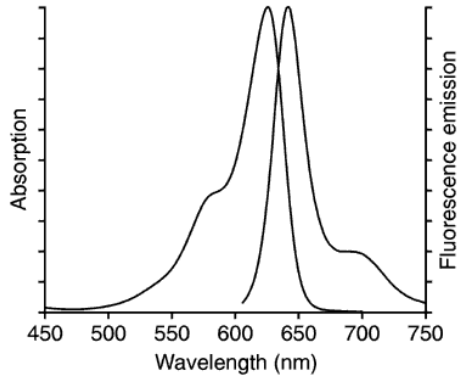
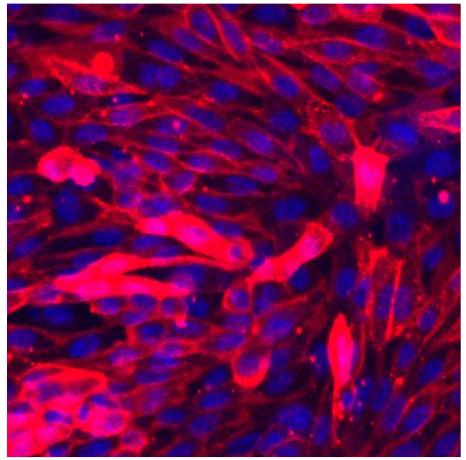
- **What excitation and emission wavelengths and filter sets should be used?**
  - The BODIPY-630/650 fluorophore has its excitation peak at 625nm and its emission peak at 641nm
  - These peaks are close, so narrow band-pass filters are needed
  - For excitation, use a narrow-bandpass filter with a peak at 630nm
    - For laser-based systems the 633nm ruby laser is ideal for excitation



- For emission, use a narrow-band filter with a peak at 650nm
- Shorter wavelength excitation and longer wavelength emission filters can be used but signals will be weaker, and there may be cross-talk with other fluorophores

- **Cells don’t bind the fluorescent ligand**
  - Likely causes:
    - Cells are not expressing the receptor
      - Compare with cells from another source
      - Compare with cells at different growth stages
      - Use the ligand for FACS to select cells expressing the receptor
    - The receptor is not reaching the cell membrane
      - Ligands do not readily cross the cell membrane, so this is difficult to test
      - An antibody approach with fixed, permeabilised cells might confirm this
    - The receptor is unable to bind the ligand
      - Fixing the cells can prevent ligand binding – use live cells
      - Too much solvent (DMSO) in buffers can damage the cells

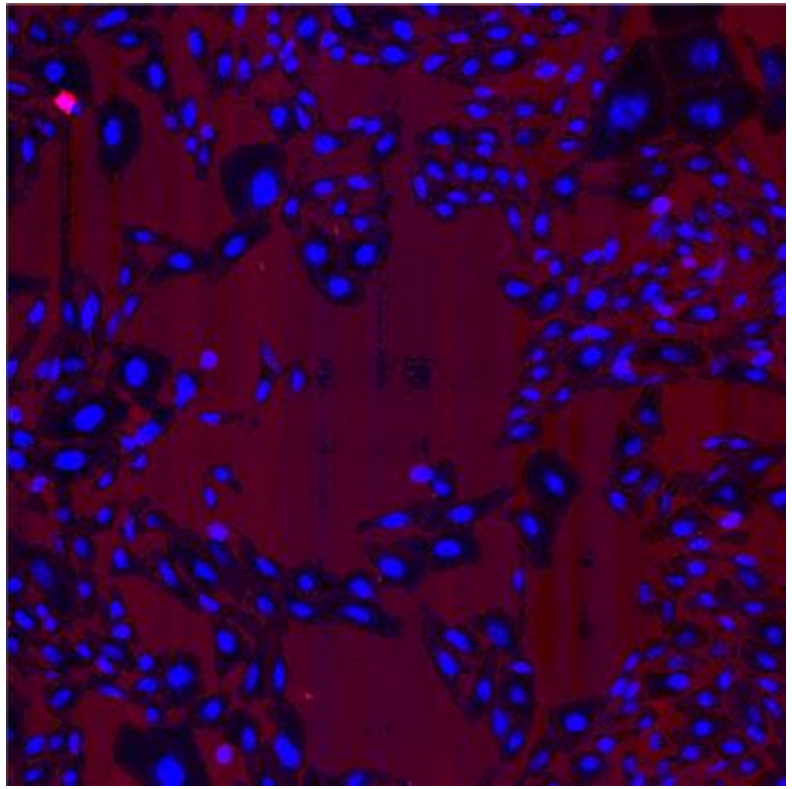
- **Cells label everywhere (cytoplasm, nucleus), not just the membrane**



  - This is likely to be because the cells are not healthy and have taken up the ligand non-specifically.
    - Check the culture conditions – cells should not be over-confluent
    - Check the assay conditions – pH should be 7.4 in isotonic buffer (eg. HBS or PBS)
    - Check there is minimal solvent used (eg DMSO < 0.1%)
    - Check the cells have not been fixed and permeabilised
  - Even in a healthy culture, there will be dead cells that show non-specific staining.
    - Make sure cell-plates are handled carefully and removed from the incubator immediately before use, not left sitting on the bench for long periods

- **Control (non target-expressing) cells label as well as the target cells**
  - Likely cause: high non-specific binding to control and target cells
    - Ensure both control and target cell lines are healthy (see previous FAQ)
    - Ensure the ligand is not being used at too high concentration (maximum should be 100nM)
  - Endogenous receptor expression by the host cell line
    - Check if the ligand is binding to an endogenous receptor expressed by the host cells by blocking with an appropriate unlabelled competitor
  - Cross-reaction to other receptors
    - the ligands may bind to other receptors
    - Use the pharmacology of the unlabelled drug from which the ligand is derived as a guide to potential additional targets to which the ligand may be binding
    - Test this with selective unlabelled blockers for the off-target receptor

- **There is very high background fluorescence**
  - BODIPY-630/650 should not show this because it's fluorescence is quenched in aqueous solution
  - If this is reported, it is likely to be due to a high concentration of the ligand in solution around the cells
    - Using the ligand at a lower final concentration (30 – 100nM gives the best signal : background).
    - Add a brief (10 min), gentle wash step to reduce the background fluorescence



- **Cells label well, and show a good saturation-binding curve**
  - but the maximal saturation binding level is lower than expected

– This may be due to the fluorescence detector reaching saturation, rather than the cells reaching binding saturation.
  - Try reducing the gain on the detector
  - Try taking measurements on an alternative instrument