Protocol for ClariTSA™ Fluorophore Kits

Please note: these products were formerly known as TSA Vivid™ Fluorophore Kits.

Introduction

ClariTSA™ reagents are exceptionally bright dyes that offer an effective way to boost signal intensity and detect low-abundance targets in spatial biology applications. They are designed for use in ISH, IHC and ICC experiments where *in situ* detection of target protein or nucleic acid sequences is required. ClariTSA™ reagents have been specifically optimized to deliver exceptional signal-to-noise in the RNAScope™ Multiplex Fluorescent v2 Assay that enables visualization of gene expression at the single cell level. They are suitable for multiplexing and can be combined with DAPI (Cat. No. 5748) counter-staining.

The basis for signal amplification comes from the specific and high-density deposition of ClariTSA™ dyes adjacent to a horse-radish peroxidase (HRP)-labeled probe, in a process termed Tyramide Signal Amplification (TSA) or Catalyzed Reporter Deposition (CARD).

Please refer to the product datasheet/certificate of analysis for information on the recommended storage conditions for ClariTSA™ products.

Protocol for *in situ* hybridization (ISH) with the RNAScope™ Multiplex Fluorescent v2 Assay

Please refer to the documentation provided with the RNAScope™ Multiplex Fluorescent v2 Assay, also available from the ACD website.

1. Reconstitute the ClariTSA™ reagent with 100 µL DMSO.

2. Recommended working dilution range: 1:750 - 1:3000. We recommend starting with a dilution of 1:1500 and adjusting based on signal intensity.

3. Apply 100 - 300 µL ClariTSA™ working solution per slide.

Keep the diluted ClariTSA™ *reagent in the dark prior to applying to slides.*

Protocols for immunohistochemistry (IHC) and immunocytochemistry (ICC)

For detailed information on preparing samples for IHC/ICC analysis please see the separate protocols available for this process. Protocols usually require further optimization. The following processes should be used as a guide only.

Appropriate controls are critical for the accurate interpretation of IHC/ICC results. All IHC/ICC experiments should include a negative control using the incubation buffer with no primary antibody to identify non-specific staining of the secondary reagents. Additional controls can be employed to support the specificity of staining

generated by the primary antibody. These include absorption controls, isotype controls (for monoclonal primary antibodies), and tissue type controls.

1. Rehydrate sections.
    - Paraffin-embedded sections should be warmed prior to deparaffinizing. First immerse in xylene then in decreasing concentrations of ethanol. Rehydrate the sections in washing buffer for 10 minutes and then drain the excess.
    - Frozen cryostat sections should be thawed at room temperature for at least 10 minutes. Rehydrate in washing buffer for 10 minutes then drain the excess washing buffer.
    - Slides containing fixed cells should be washed twice in washing buffer.

    If necessary, antigen retrieval should be performed at this point.

2. For IHC only: surround tissue with a hydrophobic barrier using a barrier pen.

3. Block non-specific staining between the primary antibodies and the tissue by incubating in blocking buffer for 30 minutes at room temperature.

4. Apply primary antibodies according to manufacturer's instructions. For fluorescent IHC staining of paraffin-embedded tissue sections using R&D Systems primary antibodies, it is recommended to incubate overnight at 2-8 °C. This incubation regime allows for optimal specific binding of antibodies to tissue targets and reduces non-specific background staining. These variables may need to be optimized for your system.

5. Wash slides/coverslips 3 times for 15 minutes each in washing buffer.

6. Apply HRP-conjugated secondary antibody for 30 minutes at room temperature.

7. Reconstitute the ClariTSA™ reagent with 100 µL DMSO.

8. Prepare ClariTSA™ working solution by diluting in amplification buffer (RNAScope™ TSA Buffer Pack, ACD Cat. No. 322810). The recommended working dilution range is 1:750 - 1:3000. We suggest starting with a dilution of 1:1500 and adjusting this based on signal intensity.

    Keep the diluted ClariTSA™ *reagent in the dark prior to applying to slides.*

9. Apply 100 - 300 µL ClariTSA™ working solution per slide for 2-10 minutes at room temperature.

10. Wash slides 3 times with washing buffer.

11. Apply counterstain (e.g. DAPI, Cat. No. 5748) and mounting media following manufacturer instructions.