

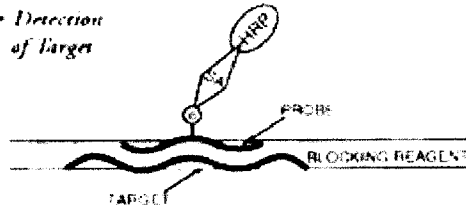
D. TSA Diagrams

The following illustration depicts **TSA-Indirect** as applied to chromogenic in situ hybridization.

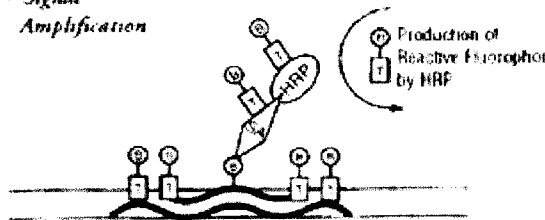
FIGURE 1

In Situ Hybridization TSA INDIRECT

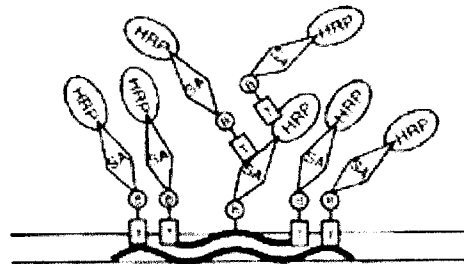
• *Detection of target*



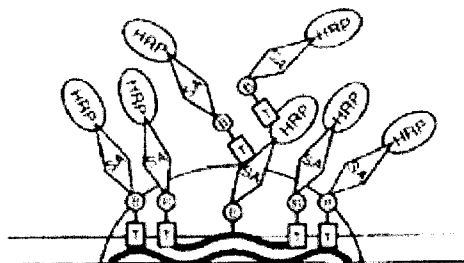
• *Signal Amplification*



• *Detection of Amplified Signal*



• *Visualization*



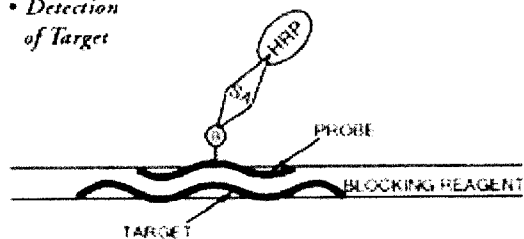
Amplification and Visualization Procedures	Actual Time Added to Protocol
Blocking Step	30 min
Wash Step	3x5 min
Incubation of Biotinyl Tyramide	3-10 min
Wash Step	3x5 min
Addition of Enzyme Conjugate	30 min
Wash Step	3x5 min
Incubation of Chromogen	

The following illustration depicts **TSA-Direct** as applied to fluorescence in situ hybridization.

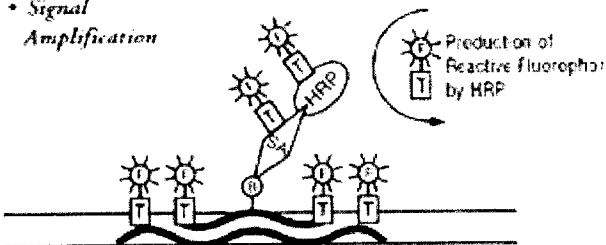
FIGURE 2

In Situ Hybridization TSA DIRECT

• *Detection of Target*



• *Signal Amplification*



• *Visualization Via Fluorescent Microscopy*

Amplification and Visualization Procedures	Actual Time Added to Protocol
Blocking Step	30 min
Wash Step	3x5 min
Incubation of Fluorophore Tyramide	3-10 min
Wash Step	3x5 min

- B = Biotin
- F = Fluorophore
- T = Tyramide
- SA = Streptavidin
- HRP = Horseradish Peroxidase
- AP = Alkaline Phosphatase

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