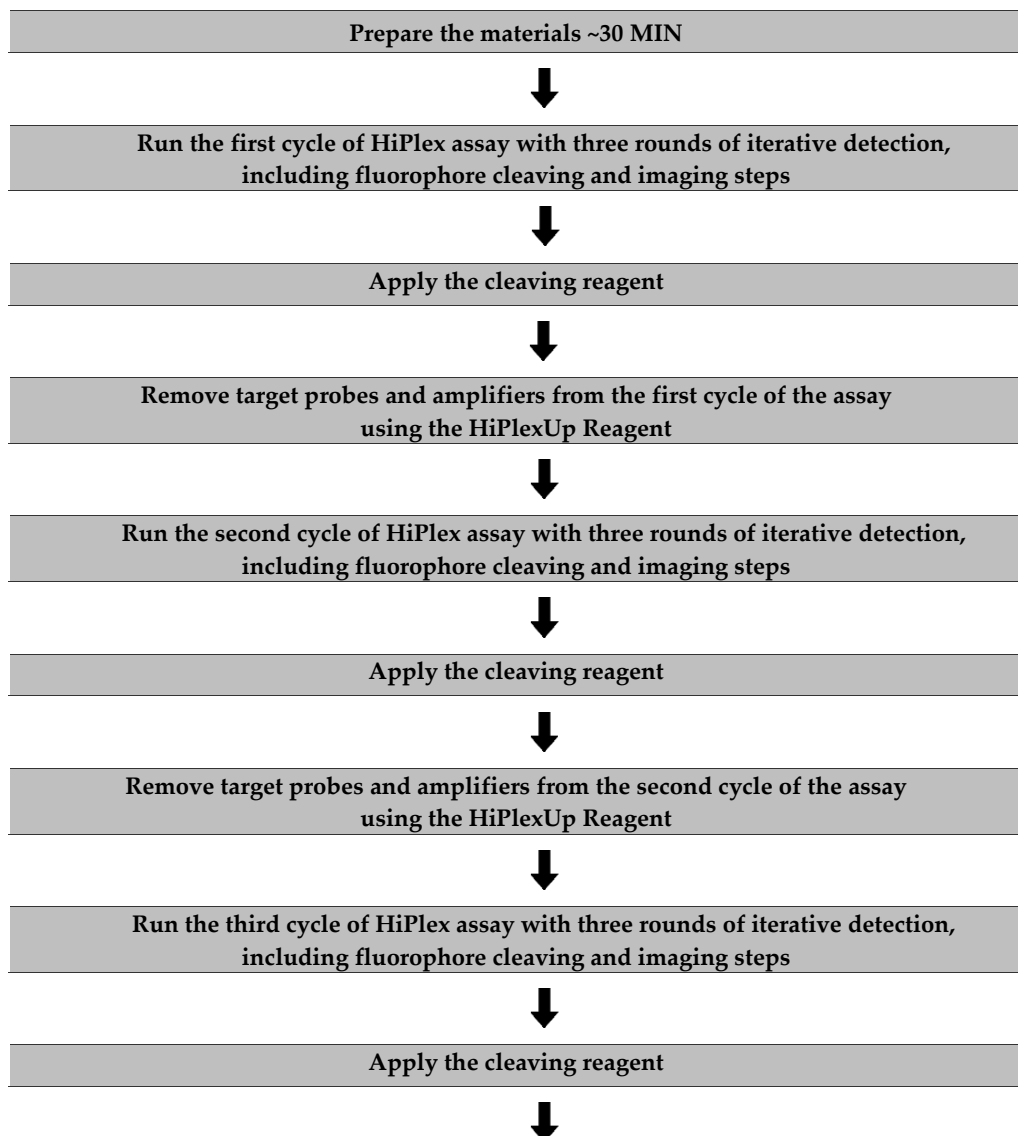


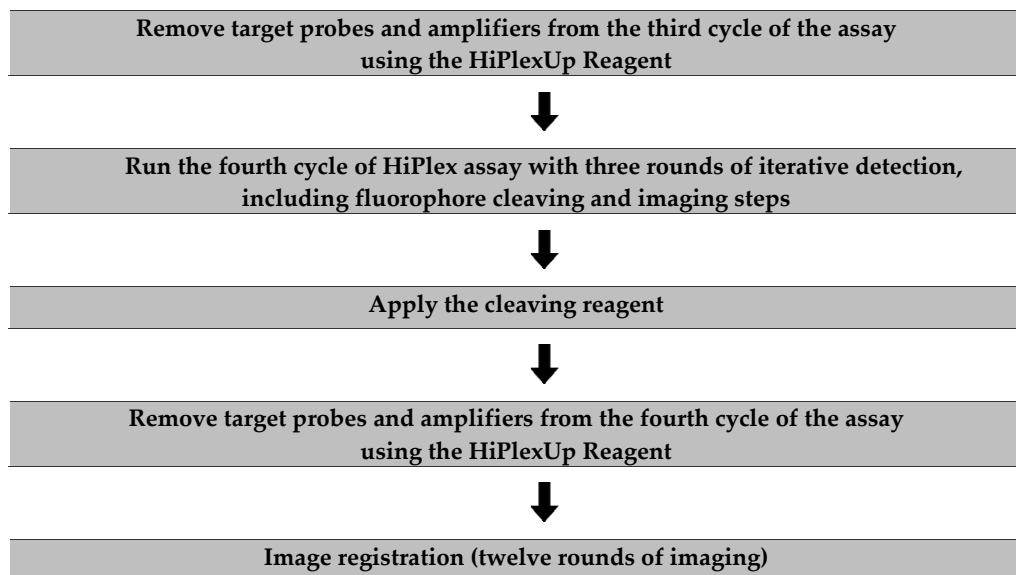
Multiplex Assays Using the HiPlexUp Reagent

Introduction

This quick guide provides instructions for using ACD's proprietary HiPlexUp Reagent, which removes target probes and amplifiers used in the RNAscope® HiPlex Assay with minimal damage to tissue RNA. Using this reagent, you can detect up to 48 target probes on the same tissue sample. Refer to the *RNAscope® HiPlex User Manual* (Doc. No. 324100-UM) for sample preparation, pretreatment, target probe hybridization, and amplification steps. Following the first cycle (three rounds) of target detection and sample imaging (see Chapter 4 of the user manual), continue with the steps listed in this quick guide to remove probes and amplifiers from your sample. Use the same procedure before performing each cycle of the HiPlex Assay described in the user manual. Refer to the Safety Data Sheet (SDS) available on the ACD website.

RNAscope® HiPlexUp Assay (48-plex) Workflow





Note: For 24-plex detection, register six rounds of imaging after completing two cycles of the HiPlex Assay. For 36-plex detection, register nine rounds of imaging after completing three cycles of the HiPlex Assay.

IMPORTANT! HiPlexUp Reagent is NOT compatible with HiPlex FFPE Reagent. If aimed at plexing number of more than 12-plex, FFPE reagent must be skipped.

Use the HiPlexUp Reagent

Workflow Steps	
PREPARE MATERIALS ~30 MINUTES	<p>Materials required:</p> <ul style="list-style-type: none"> · HiPlexUp Reagent (RNAscope® X-Plex Detection Kit) · Distilled water · Tissue-Tek® Staining Dish · Tissue-Tek® Vertical 24 Slide Rack · Cleaving reagent <ul style="list-style-type: none"> · Prepared sections · 20X SSC · HybEZ™ Humidifying System · Paper towel or absorbent paper <ol style="list-style-type: none"> 1. Prepare 5 L of 1X Wash Buffer by adding 4.90 L distilled water and 100 mL of 50X Wash Buffer to a large carboy. Mix well. <p>Note: If precipitation occurs in the 50X Wash Buffer, warm it up at 40°C for 10-20 MIN before making 1X Wash Buffer. 1X Wash Buffer may be prepared ahead of time and stored at room temperature for up to 1 month.</p> <ol style="list-style-type: none"> 2. Prepare 4X SSC by diluting one volume of 20X SSC with four volumes of distilled water. Mix well. <p>Note: If necessary, you can prepare 20X SSC by dissolving 175.3 g of NaCl and 88.2 g of sodium citrate in 800 mL of distilled water and adjusting the pH to 7.0 with a few drops of 1M HCl. Use water to adjust the volume to 1 liter. Sterilize by autoclaving or filtering under vacuum.</p>

<p>REMOVE COVERSLEIPS ~30 MINUTES</p>	<p>IMPORTANT! Perform this procedure in between each cycle of the HiPlex Assay.</p> <hr/> <ol style="list-style-type: none"> 1. Remove coverslips by soaking the slides in 4X SSC at RT for at least 30 MIN or until coverslips fall off the slides easily. If the slides are dried completely, you may need to soak the slides longer (2–3 hours or overnight). <hr/> <p>IMPORTANT! Use only 4X SSC buffer. To reduce tissue damage, do not remove the coverslips by force. Soak the slides in 4X SSC until the coverslips can be moved easily.</p> <hr/> <ol style="list-style-type: none"> 2. Wash slides briefly in 4X SSC.
<p>APPLY CLEAVING REAGENT ~40MINS</p>	<ol style="list-style-type: none"> 1. Break open a FRESH glass ampoule of provided cleaving stock solution V2. 2. Prepare a 10% cleaving solution V2 by diluting with 4X SSC. 3. Remove excess liquid from the slides while keeping them locked in the ACD EZ-Batch Slide Holder. Insert the slide holder into the HybEZ Humidity Control Tray. 4. Apply enough freshly prepared 10% cleaving solution V2 to entirely cover each section. 5. Close the tray and incubate for 15 MIN at RT. 6. Pour at least 200 mL PBST (0.5% Tween) into the transparent ACD EZ-Batch Wash Tray. 7. Place the slide holder into the wash tray and wash the slides for 2 MIN at RT. 8. Repeat the wash step one more time with fresh PBST (0.5% Tween) for 2 MIN at RT. 9. Repeat Steps 1–8.
<p>APPLY HIPLEX UP BUFFER ~15 MINUTES</p>	<ol style="list-style-type: none"> 1. Remove excess liquid and apply ~4 drops HiPlexUp Reagent to completely cover the tissue. 2. Load slides into the HybEZ™ Slide Rack, place the rack in the HybEZ™ Humidity Control Tray, close tray and incubate for 5 MIN at RT. 3. Repeat steps 1 and 2 two more times.
<p>WASH SLIDES IN 1X WASH BUFFER ~5 MIN</p>	<ol style="list-style-type: none"> 1. Wash slides in fresh 1X Wash Buffer for 2 MIN at RT with occasional agitation. 2. Repeat with fresh 1X Wash Buffer.
<p>PROCEED IMMEDIATELY TO THE NEXT CYCLE OF THE HIPLEX ASSAY (target probe hybridization, amplification, and detection)</p>	

Troubleshooting

For troubleshooting information, please contact technical support at support.acd@bio-techne.com

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