Technical Guide: Strep-Tactin XT Probes

Product Name: Strep-Tactin XT Probes

Product No: 160033

Description: Gator Strep-Tactin XT Probes are coated with Strep-Tactin XT for capture of targets with Twin-Strep-Tag.

Contents: 1x Tray of 96 Probes

Compatibility

Sample compatibility	Gator® Strep-Tactin XT probes capture targets containing Twin-Strep-Tag® (TST) (seq: SA-WSHPQFEK-$(GGGS)_2$-GSA-WSHPQFEK) with pM affinity. The high affinity capture allows for subsequent kinetic analysis of peptides and proteins. Strep-Tactin XT probes can also bind some targets with Strep-Tag®II (seq: SA-WSHPQFEK). The effectiveness of the capture will be sample-dependent and based on the accessibility of the tag. In some cases, the lower affinity capture of Strep-TagII on Strep-Tactin XT is not effective for BLI kinetics analysis applications. For an alternate method to capture Strep-TagII targets, inquire with your Field Application Scientist (FAS) or support@gatorbio.com for custom Anti-Strep-TagII probes. These probes do not bind the original Strep-Tag (seq: AWRHPQFGG) effectively due to steric/sequence mismatches. It is not recommended to analyze analytes containing biotin tags on the Strep-Tactin XT probes as they may experience some cross-reactivity towards the surface.
Surface considerations	Strep-Tactin XT probes are coated with a 3-dimensional layer of Strep-Tactin® XT on the Gator XT optics design. The probe surface contains BSA as a blocking component. If working with albumin-binding samples, please contact your FAS or support@gatorbio.com for BSA-free options.
Tag considerations	The Strep-Tactin XT capture molecule requires a specific structural conformation and pocket accessibility of the TST or Strep-TagII to achieve its high-affinity (nM–pM) interaction. If a tag variant is sterically blocked, C-terminally modified, or lacks the full N-/C-terminal pocket-fitting geometry, Strep-Tactin XT binding will fail. If this occurs, Gator Bio offers custom Anti-Strep-TagII probes (inquire with your FAS or support@gatorbio.com). The Anti-Strep-TagII antibody biosensor surface can capture the linear peptide epitope of TST or Strep-TagII directly when the tags may be sterically hindered.
Sample Media & Buffers	Gator Q Buffer (50 mL, PN: 120010; 1000 mL, PN: 120071) and K Buffer (50 mL, PN: 120011; 1000 mL, PN: 120072) are recommended for assays using Strep-Tactin XT probes. Different buffer conditions may impact the regeneration recovery over subsequent cycles and are recommended to be validated prior to the final assay. When using crude media, for example cell lysate in Expi293, a dilution into Q buffer may help to minimize background signal. Use of crude media may also impact the regeneration recovery over cycles and is recommended to be optimized prior to running the final assay.
Regeneration	Gator Regen Buffer (No Salt), PN: 120063 (sold separately) is supported for regeneration of Strep-Tactin XT probes under standard buffer conditions, allowing up to 10 regeneration cycles. Caution: Do not use NaOH-based solutions to regenerate Strep-Tactin XT probes. Strep-Tactin XT columns tolerate regeneration with basic solutions, but NaOH degrades the probe's biosensor surface over time.

Regeneration and Recapture

Strep-Tactin XT probes are regenerable using **Gator Regen Buffer (No Salt), PN: 120063** (sold separately) for up to 10 recapture cycles. In addition to the Regen Buffer (No Salt), a dedicated well is required for a neutralization buffer which should be the same composition as your assay buffer. The reagents needed for regeneration can be set up in the assay plate (96 or 384 well) or MAX plate using typical volumes. The regeneration reagents can be re-used for up to 10 recapture cycles.

It is recommended to validate capture and regeneration with new samples prior to performing a full assay using the protocol below. Prior to the first sample analyzed, pre-condition the probe for best consistency.

Standard Regeneration and Recapture Protocol

1. **Regeneration:** Regen Buffer (No Salt) 5 s, 1000 rpm
2. **Neutralization:** Assay buffer 5 s, 1000 rpm
3. Repeat steps 1-2 for a **total of 3 regeneration cycles**.
4. **Baseline:** Assay buffer, 300 s, 1000 rpm
5. **Loading:** TST-tagged capture molecule, 60-600 s, 1000 rpm
6. Repeat steps 1-5 to assess ligand regeneration and recapture recovery over multiple cycles.

If the standard regeneration protocol is insufficient, the following variations can be explored:

- Increasing the number of regeneration cycles, for example from 3 to 6 cycles
- Increasing the time of the regeneration step, for example from 5 s to 10 s

Quantitation

Strep-Tactin XT probes support one-step direct quantitation of TST-tagged targets over a typical range of 1 — 200 µg/mL using standard detection protocol, and detection down to 0.1 µg/mL using the high sensitivity protocol. The exact quantitation dynamic range will depend on the TST-tagged sample properties such as molecular weight, steric hindrance and accessibility of the TST tag.

Standard Curve Preparation

Prepare a 2-fold (2x) or 3-fold (3x) serial dilution series of the TST-tagged standard (target with known concentration) in the same matrix as your samples across the expected quantitation range. 3x dilution series can be advantageous to use for initial method development, while 2x dilution series are recommended when targeting high precision over a narrower dynamic range. At minimum 6 concentrations are recommended in addition to always including a zero concentration (blank) as a reference.

Quantitation Protocol (Single-Use)

Standard

Set temperature: 30°C

1. **Pre-wet:** Assay buffer 600 s, 1000 rpm
2. **Quantitation:** TST-tagged standard or sample, 60-120 s, 400 rpm

High Sensitivity

Set temperature: 30-37°C

1. **Pre-wet:** Assay buffer 600 s, 1000 rpm
2. **Quantitation:** TST-tagged standard or sample, 300 s, 1000 rpm

Quantitation Protocol with Regeneration

Standard

Set temperature: 30°C

1. **Pre-wet:** Assay buffer 600 s, 1000 rpm
2. **3x Regeneration cycles (pre-conditioning):**
    1. **Regeneration:** Regen Buffer (No Salt) 5 s, 1000 rpm
    2. **Neutralization:** Assay buffer 5 s, 1000 rpm
3. **Quantitation:** TST-tagged standard or sample, 60-120 s, 400 rpm
4. 3x Regeneration cycles:
    1. **Regeneration:** Regen Buffer (No Salt) 5 s, 1000 rpm
    2. **Neutralization:** Assay buffer 5 s, 1000 rpm

Repeat steps 3-4 for all standards and samples.

High Sensitivity

Set temperature: 30-37°C

1. **Pre-wet:** Assay buffer 600 s, 1000 rpm
2. **3x Regeneration cycles (pre-conditioning):**
    1. **Regeneration:** Regen Buffer (No Salt) 5 s, 1000 rpm
    2. **Neutralization:** Assay buffer 5 s, 1000 rpm
3. **Quantitation:** TST-tagged standard or sample, 300 s, 1000 rpm
4. **3x Regeneration cycles:**
    1. **Regeneration:** Regen Buffer (No Salt) 5 s, 1000 rpm
    2. **Neutralization:** Assay buffer 5 s, 1000 rpm

Repeat steps 3-4 for all standards and samples.

Notes:

- For best consistency it is recommended to include **a full pre-conditioning cycle (including TST-target binding + regeneration)** prior to the first standard/sample to be analyzed on the probes.
- Even if the probes are pre-wet in assay buffer offline, it is recommended to keep the 600 s pre-wet step to allow the probes and sample plates to reach the final set steady-state temperature. This will allow for best consistency in binding rate measurements.

Kinetics Characterization

With high affinity capture of the TST tag to Strep-Tactin XT, these probes are ideal for kinetic characterization or kinetic screening across a broad affinity range. In most cases, regeneration will effectively remove the full ligand-analyte complex from the capture surface, allowing for recapture of up to 10 TST-tagged ligands per probe in a single assay. It is recommended to validate regeneration conditions on specific samples prior to full assay implementation.

Standard kinetics assay protocol (with regeneration):

1. **Pre-wet:** Assay buffer 600 s, 1000 rpm
2. **3x Regeneration cycles (pre-conditioning):**
    1. **Regeneration:** Regen Buffer (No Salt) 5 s, 1000 rpm
    2. **Neutralization:** Assay buffer 5 s, 1000 rpm
3. **Baseline:** Assay buffer, 120-300 s, 1000 rpm
4. **Loading:** TST-tagged capture molecule, 60-600 s, 1000 rpm
5. **Baseline:** Assay buffer 30-120 s, 1000 rpm
6. **Association:** Analyte 120-600 s, 1000 rpm
7. **Dissociation:** Assay buffer 120-600 s, 1000 rpm
8. **3x Regeneration cycles:**
    1. **Regeneration:** Regen Buffer (No Salt) 5 s, 1000 rpm
    2. **Neutralization:** Assay buffer 5 s, 1000 rpm
9. Repeat steps 3-8 for additional recapture assays

Best Practices

Control loading density

Avoid saturating the probe surface with ligand. Lower loading levels reduce mass-transport limitations and prevent rebinding artifacts during dissociation. For most protein-protein analysis applications, target 0.5-1.0 nm shift loading height.

Optimize based on analyte

- **Multivalent analytes (e.g., IgG, multimers):** Reduce loading concentration/time, aim for less than 0.5 nm ligand load.
- **Small analytes (<15 kDa, peptides, fragments):** Increase TST-ligand loading concentration or extend loading time to improve signal resolution; reduce assay temperature to 20 °C on Gator Plus+ or Gator Pivot (if applicable) to maximize detection of fast-on/fast-off kinetics.

Match sample matrix

Prepare standards in the same buffer as your samples (e.g., culture media, lysate) to account for refractive index differences and reduce nonspecific binding artifacts. Equilibrate probes in sample matrix before starting the assay.

Implement reference subtraction

Include a double reference using reference probes (negative-control ligand-loaded probes, same analyte) and reference samples (target ligand-loaded probes, buffer instead of analyte) to subtract background signal and baseline drift from specific binding measurements.

Note that for Strep-Tactin XT probes in particular it is recommended to include a negative control ligand on a reference probe instead of an unloaded probe surface as the reference probe control, as the binding pocket on Strep-Tactin XT is hydrophobic and can attract non-specific binding signal to the open capture site.

Validate regeneration

Verify that regeneration fully removes bound material while maintaining capture performance across cycles by running replicates.

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