Tagify™ Custom-loaded Transposase

User Guide

V20251107

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Available Ancillary Reagents

Table 1. Available Ancillary Reagents

Component	Reference	Volume	Description	Storage
Modified Tn5 Storage Buffer	300145	1.5 ml	For diluting custom-loaded transposase reagent	Ambient
3X Coding Buffer	101284	1.5 ml	Tagmentation reaction buffer	Ambient
X Solution	101285	1.2 ml	Buffer for denaturation and removal of transposase	Ambient
MAGWise Paramagnetic Beads	101003	5 ml	Purification beads	4°C

Ancillary reagents are available in several volume formats to fit various throughput needs. Contact sales@seqwell.com to inquire about available volumes.

User-Supplied Reagents, Equipment, Consumables, and Thermal Cycler Program

Required Reagents

- 100% Ethanol (molecular biology grade)
- 10 mM Tris-HCl, pH 8.0
- Ultrapure Water (PCR grade)
- Qubit™ 1x dsDNA High Sensitivity (HS) Assay Kit (ThermoFisher P/N: Q33230), Quant-IT™ PicoGreen™ dsDNA Assay Kits (ThermoFisher P/N P7589), or other validated dsDNA quantification assay
- Agilent High Sensitivity DNA Kit for the Bioanalyzer® (Agilent P/N: 5067-4626) or Genomic DNA ScreenTape Assay for the TapeStation™ (Agilent P/N: 5067-5365)

Equipment & Consumables

- Single-channel pipettors (1-20 µl, 20-200 µl, 100-1,000 µl)
- Multi-channel pipettors (1-10 µl, 10-200 µl)
- Pipette tips (low-retention barrier tips)
- Eppendorf Tubes® (1.5 ml & 2.0 ml, DNA LoBind® Tubes)
- 96-well thermal cycler (compatible with 8-tube PCR strips)
- Magnetic stand for 8-tube PCR strip
- 0.2 ml PCR 8-tube strips and caps
- Benchtop centrifuge to pulse-spin tubes and 8-tube PCR strips
- Plate centrifuge
- Vortex mixer
- Agilent Bioanalyzer or Agilent TapeStation using Genomic DNA ScreenTape

Thermal Cycler Programs (all with lid-heating set to 105˚C)

- **TAG:** 55°C for 15 minutes
  25°C hold.

- **STOP:** 68°C for 10 minutes
  25°C hold.

Before starting the procedure:

Measure and adjust input DNA concentration. Assay the DNA concentration of each sample using Qubit, PicoGreen, or other validated dsDNA assay. Adjust the input DNA concentration for each application using 10 mM Tris-HCl, pH 8.0, if necessary. DO NOT use EDTA-containing solutions (e.g., TE buffer) to dissolve or dilute input DNA because EDTA can suppress enzymatic activity. Please refer to your downstream targeted assay for determining the appropriate DNA input.

Program thermal cycler. For convenience, set-up all applicable thermal cycler programs described in the protocol before starting.

Pulse-spin kit components. Liquids can condense or shift locations inside containers during shipment or storage. Before dispensing from reagent tubes, pulse-spin in a suitable centrifuge to gather the reagents at the bottom of the tube. If the kit components freeze during shipment or storage, thaw, mix and pulse-spin before use.

Equilibrate MAGWise Paramagnetic Beads to room temperature. Bring beads to room temperature for 30 minutes before use. Vortex to thoroughly resuspend beads prior to use. To transfer volumes accurately, pipette slowly and DO NOT pre-wet pipette tips.

Check the X Solution for precipitate before use. If a precipitate is visible, incubate at 37°C for 5 minutes (or longer). Mix gently by inversion until the precipitate dissolves (**DO NOT** vortex).

***NOTE**: X Solution contains SDS and will precipitate if stored below room temperature. Overly vigorous mixing will cause foaming.*

Please note that the 3X Coding Buffer is viscous. Store 3X Coding Buffer at room temperature. To transfer volumes accurately, pipette slowly and do not pre-wet pipette. While adding 3X Coding Buffer to reactions, mix completely by slowly pipetting up and down several times with the same pipette tip(s) used for addition. Always change pipette tips before adding 3X Coding Buffer to different reactions.

Prepare 80% ethanol fresh daily. Volume required will depend on the number of samples processed.

Prepare 10 mM Tris-HCl, pH 8.0. Prepare 10 mM Tris-HCl, pH 8.0 from a concentrated stock solution diluted with ultrapure water (molecular-biology grade).

Custom-Loaded Transposase Reagent Example Protocol

This protocol describes a general use of the Custom-Loaded Transposase Reagents. Individual use, including DNA input amounts, volumes required, and incubation times/temperatures may require optimization depending on application. **Please refer to your supplied Certificate of Analysis (CoA) for specific input amounts, volumes, and sizing expectations.**

1. Tagging Reaction

a. Pulse-spin the Custom-Loaded Transposase Reagent in a centrifuge.

b. To a new tube labeled REACTION TUBE, set up the tagging reaction by adding the following, **in order**, mixing after each addition by pipette up and down (≥10x at the transfer volume) slowly.

NOTE: *The 3X Coding Buffer is viscous; pipette carefully to avoid introducing excessive bubbles. Add 3X Coding Buffer last to prevent premature DNA condensation.*

Reagent	Volume
Custom-Loaded Reagent	Variable*
Genomic DNA	Variable*
3X Coding Buffer	1/3 of Total Volume
Total Volume	Variable

NOTE*: *For a recommended starting point of transposase reagent amount and DNA input, please follow the volumes outlined in the specific custom-loaded CoA.*

c. Close the REACTION TUBE, gently vortex the tube, pulse-spin, and transfer the REACTION TUBE to a thermal cycler and run the **TAG** program below, with lid-heating set to 105°C:

55°C for 15 minutes
25°C hold

d. Once the program is complete, proceed directly to the next step.

2. Stop Reaction

a. Pulse-spin the REACTION TUBE in a centrifuge and carefully open the tube.

b. To the REACTION TUBE, add the following volume of X Solution to each well in use. Mix thoroughly and slowly by pipetting up and down (10 times at X Solution volume), being careful not to introduce excessive bubbles.

***NOTE**: This solution contains SDS and vigorous mixing will cause it to foam. Pipetting slowly and under the surface of the solution will give the best results.*

Reagent	Example
X Solution	1/3 of Total Volume
Total Volume	Variable

c. Securely reseal and pulse-spin the REACTION TUBE, then transfer to a thermal cycler and run the **STOP** program, below, with lid-heating set to 105°C:

68°C for 10 minutes
25°C hold

d. Once the program is complete, proceed directly to the next step.

3. Tagged DNA Purification

The following recommendations are only for the use of MAGwise beads. If using other purification beads, please refer to their specific purification recommendations

a. Vortex room temperature **MAGWise Paramagnetic Beads (MAGWise**) to ensure that the beads are fully resuspended.

b. Remove the sample(s) from the thermocycler, pulse spin the REACTION TUBE, and carefully open the tube.

c. Add 1.2X equivalent volume* of **MAGWise Paramagnetic Beads** to each sample and mix thoroughly by pipetting up and down 10 times. Incubate on-bench for ≥5 minutes to allow DNA to bind.

***NOTE:** *MAGWise Beads volume ratio can be changed based on application to target different size-selections.*

d. Place the REACTION TUBE on a magnetic stand and let the beads pellet completely (≥2 minutes). A bead pellet should form on the inner walls of each tube or well and the supernatant should be visibly clear.

e. Remove and discard supernatant with a pipette. Be careful not to disturb the pellet.

f. Wash beads with 80% ethanol:

i. With the REACTION TUBE still on the magnetic stand, add enough freshly prepared 80% ethanol to cover the bead pellet to each well without disturbing the beads.

ii. After ≥30 seconds, remove and discard the supernatant being careful not to dislodge the bead pellet.

DO NOT air dry bead pellets or DNA recovery may be compromised.

iii. Repeat the previous steps (Steps 3f.i and 3f.ii) for a total of 2 washes with 80% ethanol. Remove and discard the supernatant.

iv. Cap the REACTION TUBE and remove from magnetic stand; pulse spin and return to magnetic stand to let the beads pellet again (<30 seconds). **DO NOT** air dry bead pellets. Remove any residual ethanol at the bottom of the tube.

Proceed immediately to the next steps through Tris addition.

g. Add 20µl* of 10 mM Tris-HCl, pH 8.0 to each sample. Remove the REACTION TUBE from the magnetic stand and pipette the solution along the inner wall of the tube or multiple times to thoroughly resuspend the bead pellet.

NOTE: **Product elution volume is dependent on application and desired product concentration, and thus may vary based on downstream use. Ensure that your elution volume is sufficient to account for recommended dead volume (2 µL) and for QC purposes, typically at least 2-3 µl.*

h. Incubate at room temperature for ≥5 minutes to elute the purified DNA off the beads.

i. Return the tube to the magnetic stand and allow beads to pellet on the inner walls of the wells (~2 minutes).

DO NOT air-dry bead pellets or DNA recovery may be compromised.

j. When the supernatant has completely cleared, carefully transfer 18µl* of DNA eluate from each sample to a fresh tube. The transferred supernatant contains the purified, tagged DNA product.

NOTE: **Product elution volume is dependent on application and desired product concentration, and thus may vary based on downstream use. It is recommended to transfer at least 2 µl less than the elution volume to avoid transferring beads. We also recommend saving enough volume to QC, typically at least 2-3 µl.*

SAFE STOPPING POINT

Proceed immediately with Tagged Product QC
or store purified Tagged Product at -20°C.

Tagged DNA QC & Quantification

For each set of samples processed, check tagged DNA size and quantity.

a. Quantify tagged DNA from samples using Qubit, PicoGreen DNA Assay (recommended), or similar assay.

b. Evaluate tagged DNA fragment sizes on an Agilent DNA Bioanalyzer Chip (recommended) or Agilent TapeStation using the genomic DNA ScreenTape following the manufacturer's instructions. Compare to the data provided in the custom-loaded CoA.

Please contact support@seqwell.com for more information
or for sizing optimization recommendations.

SAFE STOPPING POINT
Proceed immediately with downstream application protocols
or store purified tagmented DNA at -20°C.

Technical Assistance

For technical assistance, contact seqWell Technical Support.
Email: support@seqwell.com

Protocol Revision History:

Version	Release Date	Prior Version	Description of changes
V20251107	**November 7, 2025**	**N/A**	**First version**

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