Parse Evercode WT kit summary & user guidelines

This summary is meant to give a general idea of sample requirements and kits available for Parse Evercode WT. Additional kits and information may be available and for more information we recommend consulting the manufacturer's webpage, found here.

Parse Evercode WT is based on split-pool combinatorial barcoding and is available in multiple versions with different target numbers and samples (see table 1).

Sample preparation: In the Parse Evercode workflow the cells/nuclei are first fixed. Fixed samples can be stored at -80C, resulting in flexibility in analysis.

Sample compatibility: The technology is species agnostic and reverse transcription is performed using both a poly-dT primer and random hexamers.

Sub-libraries: After barcoding, a subset of libraries (sub-libraries) for sequencing are produced. Each sub-library contains a subset of the cells/nuclei, with samples represented proportionally to how they were included in the experiment. These can be sequenced separately.

Other capabilities: The Evercode WT kits can be used, with additional reagents, for CRISPR screens, immune profiling with BCR/TCR detection (human and mouse only) and Gene select for transcript enrichment.

Sequencing depth: A minimum sequencing depth of 20k reads/dell is recommended, but the optimal sequencing depth depends both on the specific cell type and the scientific question.

Table 1: Evercode WT kits

Evercode kit	Max cell target	Cells needed to meet max target*	Samples	Number of sub-libraries
Evercode WT Mini	10K	55K	1-12	2
Evercode WT	100K	385K	1-48	8
Evercode WT Mega	1M	3,15K	1-96*	16
Evercode WT Penta	5M		1-96**	32

*This refers to the number of cells after fixation, freezing and thawing.
** Also available in versions accommodating up to 384 samples.

Recommendation for Cells/Nuclei fixation

- Use a suitable Evercode fixation kit for sample preparation.
- Start with high quality cell/nuclei suspensions with high viability, distinct cell/nuclei. The suspension should be free of debris and have <5% aggregates.
- For assessing quality, use a viability stain such as Trypan or AO/PI.
- Do not refreeze thawed samples
- If multiplexing many samples in the same experiment (>10) please make a smaller aliquot of each sample when freezing the cells after fixation. This aliquot will be used for counting cells/nuclei prior to the run, and will not be included in the actual experiment.

Table 2: Sample fixation kits

Kit name	Input range, cells/nulcei	Sample storage stability	Theoretical max retention of cells
EvercodeTM Low Input Cell and Nuclei Fixation v3 (1-96 samples)	10k-100k	>4 months -80	65-80%
Evercode Cell fixation V3 (1-96 samples*)	100k-1m	>6 months at -80	40-60%
Evercode Nuclei Fixation v3 kit (1-96 samples*)	100k-1m	>6 months at -80	40-60%

*Fixation kits come in different formats depending on sample numbers. Up to 4 million cells/nuclei per sample if scaling up volumes (not for the low input kit).

Required Cell/nuclei numbers

See table 1 for the required number of cells needed to reach the max target of cells for the different kits. Please note that these numbers refer to the amount of cells needed **after fixation and thawing**. The total amount of cells needed for input at fixation to reach this number will be higher as there is sample loss expected at fixation, washing, counting and thawing.

- The sample retention in the fixation kits are 65-80% for the Low input version and 40-60% for the standard kit.
- Thawing of samples is associated with a 5-15% loss of cells/nuclei.

- Centrifugation speed and time should be carefully optimized for both the fresh and fixed sample to ensure maximum sample retention. NGI will use the centrifugation conditions supplied by the researcher.

- Using polypropylene tubes (low binding) is recommended.

- We highly recommend doing a pilot fixation and thawing to assess the yield.

Users guidelines:

When working with several fixed samples is recommended to have small aliquots for cell counts so actual samples are not compromised (long waiting times on ice after thawing)

Provide this information:

· Centrifugation optimization before and after fixation: speed, time, temperature

· How many samples? Target number of cells per sample?

· Did you check for aggregates after fixation, if so, were they removed?

· Cell suspension volume after fixation?

· are samples in 1.5- or 15-mL tubes? Lobind (protein low binding) or BSA treated tubes?

· Did you perform a pre-fixation QC check? RNA quality (RIN)?

· Did you store your cell at -80 °C within a Mr. Frosty Freezing Container (or equivalent device)

· Will you include control cells? How much of your experiment would you want to use for the control cells?