Biomarker Optimization on LabSat® Research

Reduce your optimization time burden with LabSat® Research

Biomarker discovery research in the field of immuno-oncology relies heavily on immunohistochemistry (IHC) assays, involving the execution of tailored protocols with a high number of steps, all of which are subject to optimization. This can be a lengthy process that can take days, weeks and even months to perform, and requires a step-by-step approach to finetune protocol conditions such as assay temperature, antibody concentration or incubation times, in order to achieve optimal results with minimal investment of resources. Turnaround times of manual assays, even for single marker stainings, often require overnight incubations, and existing automated staining platforms, while faster, still require several hours to stain one marker and can appear to be very expensive when performed for high throughput, which turns these optimization phases into a long burdensome processes.

The LabSat® Research platform is an automated tissue immunostainer device. The system is pressurized and provides precise temperature and reagent flow control in order to finetune and optimize conditions, allowing high-quality and fast multiplexing of up to 6 markers within a few hours, and can perform single-plex staining under 30 minutes. This short timeframe is particularly well suited to run fast protocols during assay optimization in just a few samples, instead of staining large batches of slides to save precious samples or reagents.

FFeX: Why is LabSat® Research so fast?

Lunaphore's core technology, the Fast Fluidic Exchange (FFeX), utilizes a microfluidic "Staining Chip" that delivers reagents sequentially onto a tissue sample. The chip is essentially a microfluidic tissue processor which forms a shallow chamber over the tissue sample. Reagents flow in and out of the chamber thanks to pressure differentials and the shallowness of the chamber increases the speed of exchange between antibodies and tissue epitopes, dramatically reducing the incubation times. Moreover, the staining chamber is filled almost instantaneously, preventing different areas of the tissue from being incubated unevenly, hence providing a great degree of signal uniformity in a controlled environment allowing more robust and reliable results. This active flow of reagents produces a fast exchange at the tissue surface, reducing the required incubation times.

1. How to start protocol optimization with LabSat® Research?

Using standard tools, the optimization process can take weeks, especially in the context of multiplexing assays. With LabSat® Research it is possible to significantly reduce that timeframe, since many different conditions can be run in a working day and sequentially, and after each run deciding which parameter to improve next. Here we present a possible workflow (Figure 1).

Workflow: single marker optimization

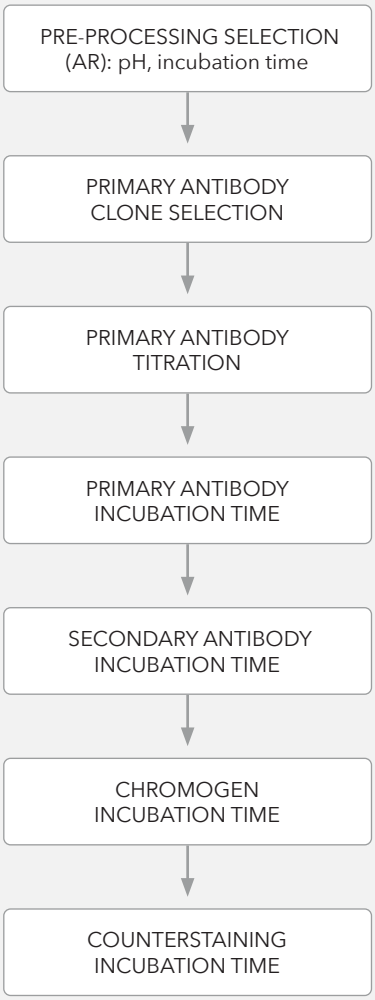

FIGURE 1 *- Proposed workflow for single marker optimization.*

Here we present two examples of optimization protocols performed on LabSat® Research.

- **One-day optimization of HER2.**
- **Three-day optimization of PR.**

CASE STUDY 1:
One-day optimization workflow of HER2

HER2 (human epidermal growth factor receptor 2) is a protein codified by the *ERBB2* gene that plays a role in the development of breast cancer, and is commonly found in multiplex panels for the study of breast cancer.

Figure 2 illustrates the steps involved in a typical one-day optimization process for HER2 IHC. In the initial stage of the process, the primary antibody (AbI) clone was chosen, followed by the titration of the AbI and its incubation time. Then, the incubation time of the secondary antibody (AbII) and the DAB were tested and selected.

This one-day process showed that with LabSat® Research it is possible to optimize four parameters in nine steps of approximately 30 minutes each.

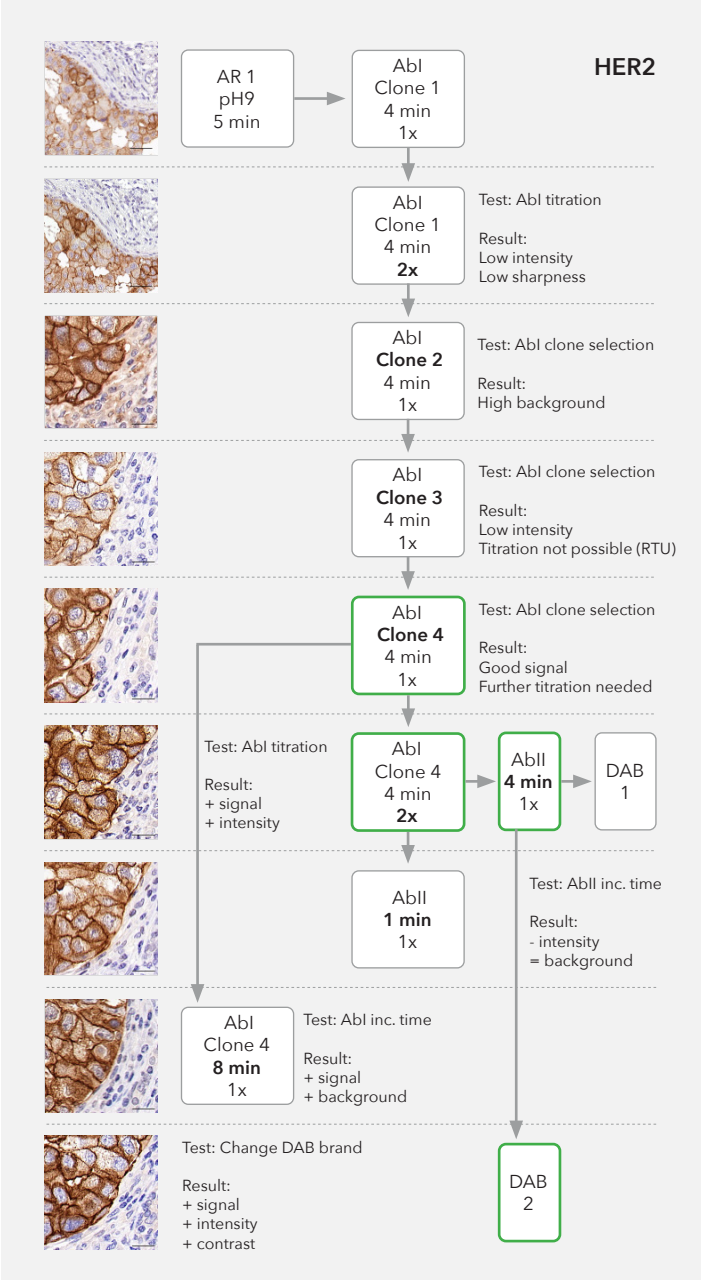

FIGURE 2 *- One-day optimization workflow of HER2 on FFPE breast cancer samples: 1) Selection of clone 4 of AbI; 2) Selection of 2x titration of AbI and 4 min incubation time; 3) Selection of 4 min incubation time of AbII and selection of DAB 2.*

CASE STUDY 2:
Three-day optimization assay of PR

Progesterone receptor (PR) is a hormone receptor found on breast cells. It is also considered a hallmark of breast cancer and generally included in multiplex panels for breast cancer research.

Figure 3 shows the steps involved in the first day of PR protocol optimization. The starting steps of this process focused on selecting the best antigen retrieval (AR) pH and incubation time.

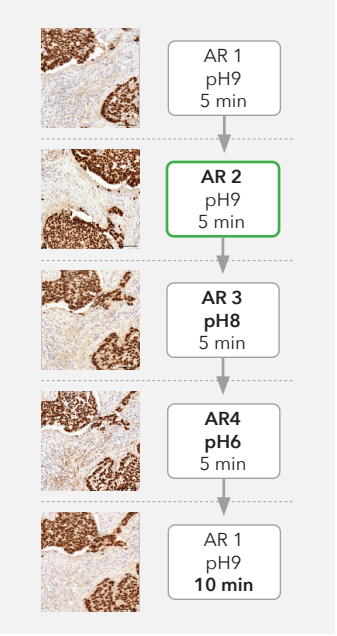

Figure 4 recaps the PR optimization steps during day 2. The selection of the incubation times of AbI and AbII took place in the initial stage of this day. The results obtained were considered satisfactory compared to the detection system reference, therefore only two conditions were tested. Subsequently, two different blocking times were assessed and finally, the last assay of the day was the test of the hematoxylin incubation time. Overall, during this second day, it was possible to test six different conditions for four parameters.

FIGURE 3 *- Day 1 of the optimization workflow of PR on FFPE breast tissue samples: 1) Selection of the AR type 2 with pH=9.*

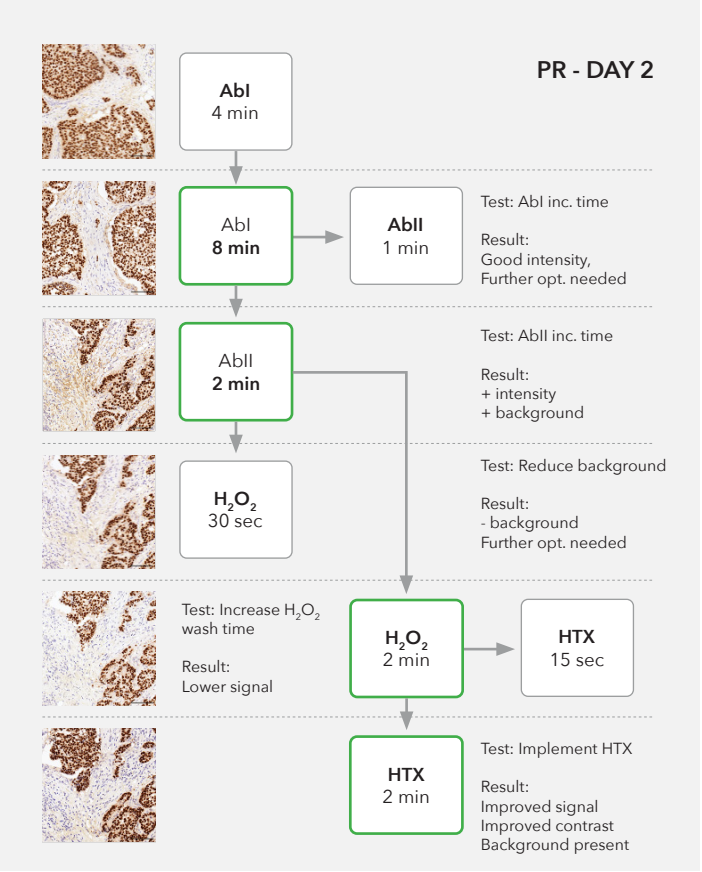

FIGURE 4 *- Day two of the optimization workflow of PR on FFPE breast tissue samples: 1) Selection of 8 min inc. time of AbI; 2) Selection of 2 min inc. time of AbII; 3) Selection of 2 min of blocking inc. time; 4) Selection of 2 min of HTX inc. time.*

The third and last day of the PR protocol optimization, as depicted in Figure 5, involved four conditions within two different parameters. The AbI concentration and incubation times were first addressed. As the final steps, the AbI incubation time was re-assessed, due to the previous titration increase, and an AbII incubation time was selected.

Overall, with a total of 17 conditions tested for seven different parameters, the 3-day process for PR optimization showed that, despite marker optimization being a long and complex process, LabSat® Research provides a simple and affordable time-saving solution.

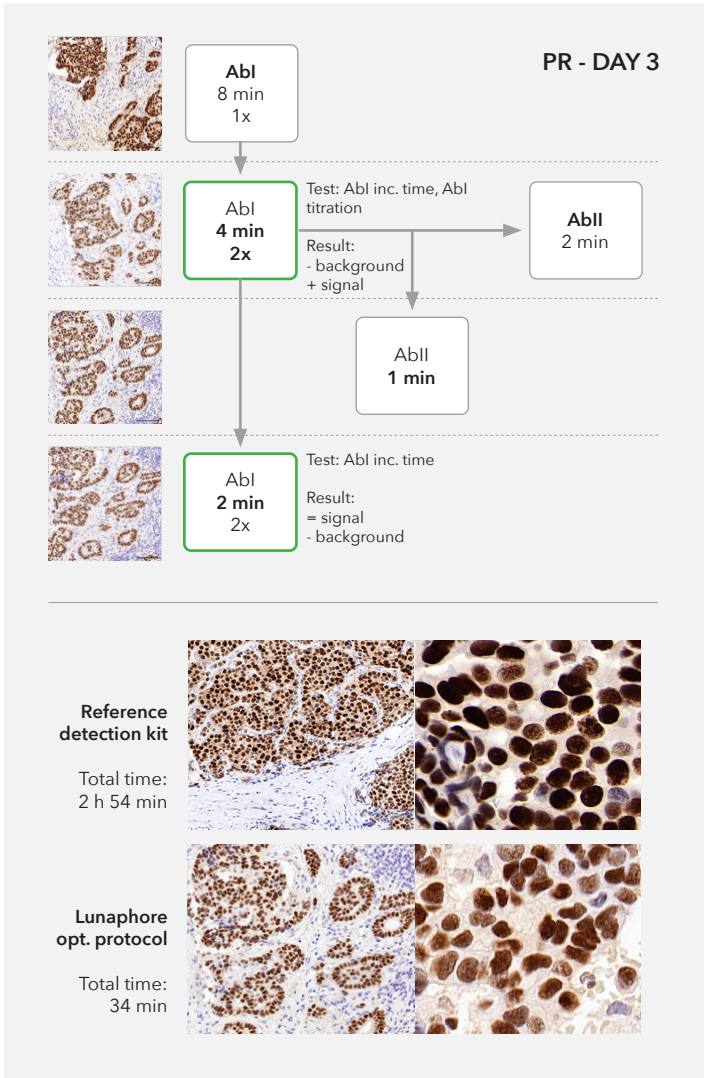

FIGURE 5 *- Day 3 of the optimization workflow of PR on FFPE breast tissue samples: Top panel:1) Selection of 2x titration of AbI; 2) Selection of 4 min incubation time of AbI and 1 min incubation time of AbII. Bottom panel: results and timing follwing optimization with LabSat® Research compared to a reference staining.*

2. Validation and quality assessment

Protocol validation is performed through a series of experiments to assess its repeatability and reproducibility, as well as the staining quality. In this model, a protocol for the estrogen receptor (ER) marker was optimized upstream.

As illustrated by Figure 6, using the optimized protocol for ER on 5 different slides and compared to a detection system of reference, staining showed equivalent high-quality results.

	AR	Block	AbI	AbII	DAB	HTX	Tot. time: **32 min**
ER	AR 2 pH9 5 min	H_2O_2 30 sec	[ ] 2x 4 min	2 min	2 min	2 min	

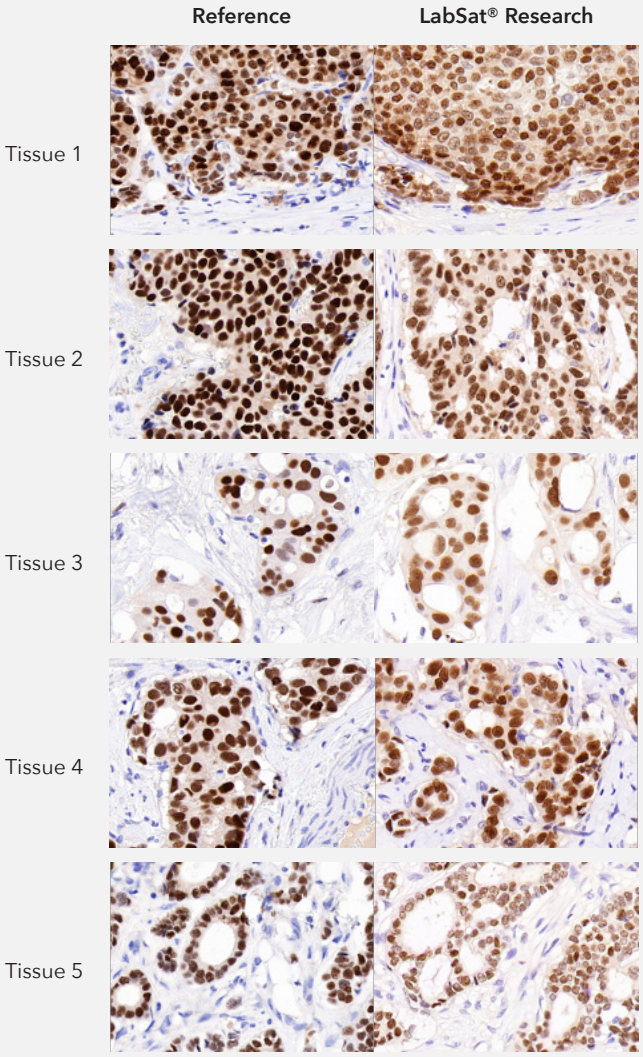

FIGURE 6 *- LabSat® Research protocol for ER applied across several FFPE tissue samples and compared to reference stainings.*

Summary and conclusion

- LabSat® Research enables ultra-fast full optimization of biomarkers.
- Optimization is possible within a day, or a few days for complex markers.
- Excellent repeatability and reproducibility for the optimized protocols can be obtained.
- Excellent results were obtained in the quality assessment, and it was deemed comparable to the gold standard reference staining.

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