



Protocol for Incubating and Freezing Samples in Smart Tubes without using a Base Station  
Protocol Number: STMTS1PNB-131103-RevC

Required Items:

Sodium Heparin Vacutainer

Smart Tubes at room temperature (one per incubation condition)

Water bath pre-warmed to 37°C

-80°C freezer or dry ice

Protocol:

1. Draw blood using approved techniques into a Vacutainer or equivalent tube containing sodium heparin. Ensure good mixing with the anticoagulant by inverting the securely capped tube at least 6 times. Keep the blood at room temperature until it is assayed with the Smart Tube system, ideally within 2 hours of the blood draw.
2. OPTIONAL: If agents (ie. drugs, cytokines, TLR ligands) are to be mixed with the blood to elicit a biological response during incubation it may be desirable to add these agents to the Smart Tubes prior to adding the biological sample. Depending on the stability of the agent and how it is added to the Smart Tube it may be added well in advance of the experiment, but can also be added during this step.
3. Add 1 milliliter of the blood sample to each Smart Tube. Immediately cap the Smart Tube and invert 6 times to ensure good mixing with the agents added.
4. OPTIONAL: Place the Smart Tubes in a 37°C water bath for the desired incubation duration to elicit the desired biological response.
5. At the end of the desired incubation duration remove the Smart Tubes from the water bath and activate the Smart Tubes manually. To activate a Smart Tube manually, make sure the cap is

screwed on securely and then bend the Smart Tube in the middle until the ampoule inside breaks, then invert the Smart Tube 10 times to ensure good mixing.

6. Incubate the activated Smart Tubes at room temperature for 10 minutes.
7. Immediately transfer the Smart Tubes to a -80°C freezer or place in direct contact with dry ice. The Smart Tubes should be stored at -80°C until the sample is analyzed. Samples frozen in Smart Tubes should not be stored at temperatures warmer than -80°C. Smart Tubes have not been validated for storage in liquid nitrogen.
8. To thaw a sample frozen in a Smart Tube please execute the protocol "Smart Tube THAW Protocol STTLNB-131103".

## Notes

The steps marked "OPTIONAL" are not required if incubation of the blood sample with exogenous agents to elicit biological responses is not a part of the experimental protocol.

The Smart Tube system has not been validated with anticoagulants other than sodium heparin. Note that some anticoagulants have a detrimental effect on calcium signaling and others may be metabolized if the sample is incubated for prolonged periods of time. It is recommended that if the user chooses to use an anticoagulant other than sodium heparin that the user validate their assay and the storage performance of the Smart Tube system with their anticoagulant of choice.

If a cell suspension (PBMC or a cell line) is used instead of whole blood an anticoagulant is unnecessary. If the cell suspension is to be incubated with an agent though to induce a biological response then the cells should be suspended in a culture media previously demonstrated to facilitate this biological response. Note that some culture media (ie. complete RPMI) contain autofluorescent components that when used with the Smart Tube System may increase the autofluorescence of the cells.

For longer incubation times (ie. >6 hours) some investigators working with whole blood have mixed the whole blood with culture media in a 1:1 ratio or even with an excess of culture media. The total volume of whole blood plus culture media added to the Smart Tube should be 1 milliliter. The culture media of interest should be selected such that it has minimal levels of compounds that could contribute to autofluorescence of the cells (ie. riboflavin).