

Internal Positive Controls: What You Need to Know

BAX® System PCR tablets are specially designed to include all reagents needed for the reaction, including primers, polymerase, nucleotides and an internal positive control (INPC). These reagents are packaged into a stable, dry, manufactured tablet to enhance the method's ease of use, reduce the opportunity for operator error and help prolong the shelf life of these reagents.

But, what is the significance of including the INPC reaction? A team of R&D experts from DuPont Nutrition & Health provides helpful insights to the importance of this process and answers some of the most frequently asked questions.

What does the INPC do?

In the BAX® System method, the INPC reaction helps to ensure that every part of the testing process runs smoothly. Among other benefits, a successful INPC reaction can notify the lab technician that:

- **The lysis reagent was properly inactivated during the lysis procedure.** If the protease is not inactivated, it can degrade the polymerase in the PCR reaction and cause a failed PCR run; without the INPC reaction for comparison, the failed PCR run may look like a typical negative result.
- **The sample preparation procedure was performed correctly.** Changes in the INPC reaction can indicate that a user error occurred in the sample preparation process, such as using the wrong lysis buffer, hydrating PCR tablets with the wrong volume of lysate or even accidentally removing the PCR tablet from the tube when pipetting or mixing samples.
- **The sample did not contain PCR inhibitors.** The INPC is specifically designed to be more sensitive to PCR inhibitors than the test reaction, so changes in the INPC response can indicate that the test result was affected by the presence of PCR inhibitors or other factors.
- **The Q7 instrument provided the correct heating and cooling cycles to amplify any target DNA present in the sample.** A positive INPC reaction is required for any negative samples to assure the user that the appropriate conditions for a successful amplification of target (had it been present) were met. Without this comparison, it could be impossible to differentiate between a failed run and a negative sample

How does the INPC work?

The INPC uses a synthetic strand of DNA to simulate a positive response within each test reaction. A synthetic DNA strand is used to eliminate any type of cross-reaction between the control response and actual test response. Because both the synthetic target and the reagents required for its amplification are included in each BAX® System PCR tablet, the synthetic DNA is expected to consistently amplify to detectable levels and produce a positive response during processing in the Q7 instrument. Furthermore, because the amount of this INPC target is pre-determined and can be reliably predicted for each PCR test, changes in the INPC response can indicate an issue within the test itself.

Because the INPC is designed to be more sensitive to PCR inhibitors than the test reaction, this control response will be affected by these external factors before the test reaction. This allows the user to rely on the INPC response to indicate that an issue may be present, without returning inaccurate results. This design has been perfected with the BAX® System through more than 14 years of development and rigorous validation testing so that the multiplex reaction is capable of amplifying both the target and control DNA without any cross-interference.

What is the biggest risk of not using an INPC?

The most significant risk of testing samples without an INPC reaction is reporting a false negative test result. This occurs when the target is present in the test sample, but for some reason the conditions for a successful amplification – including proper sample preparation, accurate instrument performance and the absence of PCR inhibitors – were not achieved. As a result, the target DNA is not detected, and the method inaccurately shows that the sample is negative for the pathogen.

If an INPC reaction is not included with each test sample, the overall results of a process run may look normal and fail to indicate that the conditions under which some samples were tested did not allow for pathogen detection. If an INPC reaction is included with each test sample, however, the highly-sensitive INPC response can signal to the operator that something may be wrong.

In addition, not including an INPC minimizes the ability to troubleshoot a failed reaction. Without the presence of INPC response to help determine the difference between a failed reaction and a false negative result, it may be impossible for a technician to address and prevent potential issues within the system or process.

Can an external positive control (EPC) provide the same benefits as an INPC?

For testing methods that do not include an INPC, an external positive control (EPC) must be run separately from the test reaction, yet provides fewer benefits.

Many methods, including non-PCR methods such as ELISA and isothermal DNA amplification, recommend that one EPC sample be run with each process run of test samples to ensure that processing steps were performed correctly. However, not all samples are created equal – even within a single run – and an EPC may not actually control for certain types of potential failures. For example, a rack of samples could include a mis-pipetting error, where a test sample is hydrated incorrectly but the EPC is prepared accurately. When these samples are processed, the EPC will indicate that the test was successful, even though the mis-pipetted sample may have returned inaccurate results.

What is the procedure for running an INPC?

Because the INPC is included within each BAX® System PCR tablet and automatically detected within the instrument, no additional steps are required in order to receive this added benefit.

If a test method does not include an INPC reaction and the positive control must be run separately from the test reaction, two separate reactions should be prepared for each test sample – one for the pathogen test and one as a positive control for that particular sample. Not only does this procedure double the sample preparation work of a single-reaction method with an included INPC, but it also reduces the number of samples that can be performed simultaneously by half, as each sample requires two spaces in the processing instrument.

Does the INPC add extra cost to each test?

The majority of the cost of an internal positive control process is in the design and development of the control itself. Because the INPC included with every BAX® System PCR tablet has been proven and perfected over years of assay development, the cost of this added benefit can be counted in pennies per test, with a priceless return in peace of mind.