

Pharmaceutical Applications

Rapid screening tests using bacteria looking for evidence of toxicity, genotoxicity and mutagenicity.

The development of new drug compounds involves the screening of a large number of both natural products and synthetic products or derivatives. These compounds are screened not only for efficacy but also for potential side effects. Some of the earliest screens involve rapid screening tests using bacteria looking for evidence of toxicity, genotoxicity and mutagenicity. To serve this need, EBPI has developed a number of sensitive screening technologies that have been incorporated into easy-to-use test kit systems. Pharmaceutical companies in the USA, Canada, Europe and Asia are currently using the tests listed below.

Toxi-ChromoTest™ Kit

This test can be completed in a three hour time period in a standard laboratory and requires only a micro pipette and a 37°C incubator. All other materials needed are supplied with the kit. The test measures the ability of a rough mutant of the E. coli test organism to successfully synthesize and excrete the enzyme beta galactosidase into the liquid medium it is growing within. If the material being screened interferes with the induction of the lacZ gene and its subsequent expression, the test will show a positive result. The test has both positive and negative control endpoints. The results expressed as a blue color in the growth medium can be read qualitatively by eye or quantitatively using a micro-plate reader at 615nm. The test is supplied with both a positive and negative control to ensure that the kit is functioning properly.

SOS-ChromoTest™ Kit

This test can be completed in a two hour time period following an initial grow-up of the test bacteria either

overnight or within four hours the day of the test. In order to run the test the user must have access to a micropipette, an incubator operating at 37°C and a meter to measure the optical density or turbidity of the bacterial suspension to be used in the test. All other required materials are supplied with the kit. The test is based on a strain of *E. coli* in which the SOS gene promoter has been linked to the LacZ gene. As a result, when there is DNA damage to the cell, which would normally result in the SOS gene repair complex being activated to repair it, the Lac Z gene is activated and beta galactosidase is excreted into the surrounding growth medium. The enzyme then reacts with a chromogenic substrate which results in a blue color being expressed. The extent to which the test material is causing the cell to try and repair DNA damage can then be read qualitatively by eye or quantitatively using a micro-plate reader at 615nm. The test is supplied with both positive genotoxic and negative genotoxic controls to ensure that the kit is functioning properly.

The SOS ChromoTest™ kit can be used with or without S-9 activation.

Muta-Chromoplate™ Kit

This test requires a five day period to complete. The test is set up on day one and the results read on day five. The user must have access to a micropipette and an incubator running at 37°C. The test itself is a microplate format of the reverse mutation 'AMES TEST.' The Salmonella strains have a mutation that does not allow them to metabolize histidine. As the bacteria under the test conditions “reverse mutate” back to the wild strain and regain their ability to metabolize the histidine present in the growth medium, the medium will turn from a purple to a yellow color. The test is set up with both a background plate to evaluate the natural rate of reverse mutation and a positive control plate where exposure to a known mutagen occurs. A control chart is also supplied so that the user can easily determine whether or not there is a statistically significant difference in the mutation rate between the background rate and the rate of mutation when exposure to the

material of interest occurs.