BIO-RAD

Checkmark™ (Ö mark) Plate Instruction
- for manual usage

PURPOSE

The Checkmark Check Plate (√ mark) is used for validating the performance of the measurement system of Bio-Rad absorbance microplate readers.
With included Checkmark PC software package, you will be able to test all parameters that Checkmark plate is designed for. However, if you do not want to use a PC for your test, the following parameters can be tested manually (or for Model 680 reader, with its on-board Checkmark protocol):
• Plate positioning
• Crosstalk
• Spectral blocking
• Linearity
• Filter wavelength
• Overflow (0% transmittance) and
• 0OD (100% transmittance)

EXPLANATION OF THE TEST

Testing the instrument involves three steps:
1. Program the test on the reader according to the Instruction Manual for Model 680 reader, make sure you key in all required plate specific reference values (given in the certificate document) for each wavelength. Once programmed the test will remain stored on Model 680 reader until changed.
2. Run the test using the √ mark plate on the reader.
3. Check the printed results against the specified limits. In the case with Model 680 reader, the result can be printed automatically or manually.

STORAGE

The √ mark Plate should be kept out of direct light and away from temperature extremes and high humidity. While not in use the plate should be stored in the case provided. Handle the plate carefully. The glass filters will break if dropped or mishandled.

CLEANING THE PLATE

If stored and handled properly, the plate should stay relatively clean with only a small accumulation of dust. Use a can of compressed tetrafluoroethane duster suitable for electronics or camera work to blow any dust particles off the glass. Do not use water or cleaning solutions of any kind as they may discolor the plate. For a more thorough cleaning send the plate back to the manufacturer.

Plate Layout:

	1	2	3	4	5	6	7	8	9	10	11	12
A	PLATE POSITIONING	CROSSTALK	CROSSTALK	SPECTRAL BLOCKING	NEUTRAL DENSITY 1	NEUTRAL DENSITY 2	NEUTRAL DENSITY 3	NEUTRAL DENSITY 4	FILTER CHECK	OVERFLOW	AIR = 0 OD	PLATE POSITIONING
B												
C												
D												
E												
F												
G												
H												

PARAMETERS TO BE TESTED

Plate Positioning:

The holes in column 1 and column 12 are used to verify correct positioning of the microplate in relation to the optical system of the readers
Criteria:
The measured values for all wells in column 1 and 12 must not exceed 0.010 OD
The test may be performed with any filter wavelength installed on the instrument.

Crosstalk:

Column 2 and 3 on the √ mark plate are used to check for crosstalk – the channel to channel influence in the measurement system. As only multi-chancel measurement systems may show crosstalk this check is only relevant for the following readers Model 550, 680, and Benchmark.
Criteria:
The measured values for the wells A2, C2, E2, G2, B3, D3, F and H3 *must not exceed 0.003 OD. All other measured values in column 2 and 3 must show overflow.*
The test may be performed with any filter wavelength installed on the instrument.

Spectral Blocking:
Column 4 of the √ mark plate incorporates an optical filter used to verify that the measurement results are not influenced by the infra-red portion of the spectrum of the light source.
Criteria:
The measured values for all wells in column 4 must show a value >= the upper limit of the indication range of the instrument tested –0.100 OD or overflow.
The test may be performed with any filter wavelength installed on the instrument in the range of 340nm to 690nm.

Linearity - Neutral Density 1 to 4:
Columns 5 to 8 are each holding 4 different neutral density filters, which may be used to verify the linearity of the measurement system in the wavelength range of 400 to 750nm.
As the verification of the linearity requires the calculation of a linear regression (and it's parameters) it is best to use the evaluation software provided on the instruments or with Checkmark PC software.

The results used to verify linearity are the:

Coefficient of Correlation for the linear regression that has to meet the following criteria:
Coefficient of Correlation   0.99 or 99%

Coefficient of Variation must not exceed the limits given for each standard in the related table 'Linearity' column 'max. CV%' shown later in this document.

Filter Check:
Column 9 of the √ mark plate incorporates a special filter giving different absorbance values for different wavelengths, steadily decreasing from the low to the high end of the relevant spectrum.
The table titled 'Filter Check' in the 'Calibration Data' section in the certificate shows the upper- (Maximum) and lower- (Minimum) limits for a number of wavelengths for your individual √mark plate, with the average of all wells in column 9.

Overflow:
Column 10 of the √ mark plate completely blocks the optical path of the measurement system and is used to verify if instrument is correctly showing overflow (transmittance = 0%, absorbance infinite).
Criteria:
The measured values for all wells in column 10 must show overflow.
The test may be performed with any filter wavelength installed on the instrument.

Air = 0 OD:
Column 11 of the √ mark plate is used to perform a measurement through air corresponding to 100% transmittance or 0 OD.
Criteria:
The measured values for all wells in column 11 must show a value in the range of –0.003 to 0.003 OD.
The test may be performed with any filter wavelength installed on the instrument.

<u>Perform the test using the instrument software</u>
Install the Checkmark software (from the CD included with Checkmark plate package) on a Windows based platform, and you can perform reader performance validation test on all of the following Bio-Rad absorbance microplate readers: Model 3550, 550, 680, Benchmark, Benchmark Plus, Ultramark and Ultramark EX readers. Place the √ mark Plate in the plate carrier of the reader. Orient the plate so that position A1 is located in the left top corner. Follow the Checkmark Software Instruction manual to perform a validation test.

All test results are dated and a record should be kept (with Checkmark PC software, all previously run tests are stored in a database; with Model 680 on-board protocol, you should print out your test result and keep it for your records). It is important to compare results regularly to give you an indication as to whether your reader is functioning properly over time.