D-Glucose Assay, UV-method
(R-Biopharm, Cat. No. 0 716 251)

modified by Maitreya Dunham and Cheryl Christianson
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UV cuvettes (Plastibrand Disposable UV-cuvette, micro, Cat. No. 7592 10)
Cuvette caps (Plastibrand Caps for Ultravette™, Cat. No. 759241)

~240 assays per kit

Preparation of Reagents:

Add 45ml MilliQ to one bottle 1 and mix until powder is completely dissolved. Equilibrate to room temperature before use (solution 1 is stable for 4 weeks at 2 to 8°C, or for 2 months at -15 to -25°C).
Suspension 2 is stable at 2 to 8°C.

Glucose-limited Media (~0.8g/L glucose):

Dilute samples 1:2
Use the following chart to add the appropriate amount of reagents to the appropriate cuvettes:

	BLANK	SAMPLE(diluted 1:2)	CONTROL
Solution 1	500µl	500µl	500µl
Sample	-	50µl	-
Control	-	-	50µl
MilliQ	1000µl	950µl	950µl

Cap cuvettes, and mix by inverting several times.
Read Absorbance @340nm after approx. 3 minutes (check for bubbles, gently knock out those that may interfere with the measurement)
Then add:

Suspension 2	10µl	10µl	10µl

Cap cuvettes, and mix by inverting several times.
Read Absorbance @340nm after approx. 15 minutes.

Control Concentration (g/L) = 0.8636 x _A (sample volume = 50µl, no dilution)
Sample Concentration (g/L) = 2 x 0.8636 x _A (sample volume = 50µl, 1:2 dilution)

Glucose-limited Filtrates:

DO NOT dilute samples, increase sample volume to 1000µl
Use the following chart to add the appropriate amount of reagents to the appropriate cuvettes:

	BLANK	SAMPLE	CONTROL
Solution 1	500µl	500µl	500µl
Sample	-	1000µl	-
Control	-	-	50µl

MilliQ	1000µl	-	950µl

Cap cuvettes, and mix by inverting several times.
Read Absorbance @340nm after approx. 3 minutes (check for bubbles, gently knock out those that may interfere with the measurement)
Then add:

Suspension 2	10µl	10µl	10µl

Cap cuvettes, and mix by inverting several times.
Read Absorbance @340nm after approx. 15 minutes.

Control Concentration (g/L) = 0.8636 x _A (sample volume = 50µl, no dilution)
Sample Concentration (g/L) = 0.04318 x _A (sample volume = 1000µl, no dilution)

Important Notes

According to the kit, _A must be greater than or equal to 0.100 absorbance units for accurate results. You can increase your sample volume up to 1000µl and decrease the amount MilliQ added in order to achieve this minimum value.
I do not trust the spec readings if they are above 1.00. If your readings are above 1.00 at the minimum sample volume you must dilute your samples (and adjust the concentration calculation accordingly).
Mix samples well and be careful of leakage since the caps do not always form a perfect seal.
Be extra careful of bubbles that may interfere with your readings. Gently knock out any bubbles before taking the absorbance measurements.
Adjust your sample values according to the difference between the actual concentration of the control solution and the calculated concentration determined using _A. (For example, if your control solution is actually 0.500g/L and the calculated value is 0.492g/L, then you must multiply 0.492g/L by 1.016260163 for it to equal 0.500g/L. Thus, you must also multiply all of your sample values by the same number.)