MicroResp™
Technical Manual

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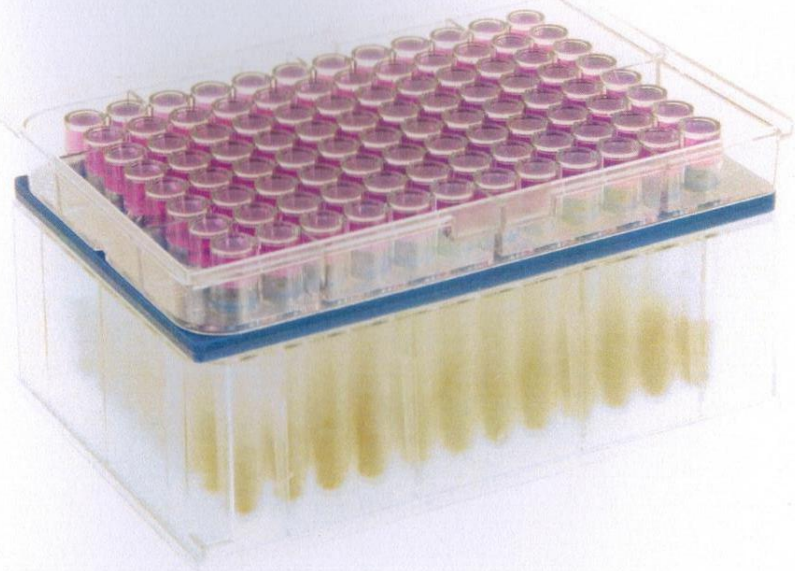
James Hutton Limited

MicroResp™
Technical Manual

A Versatile Soil Respiration System



MicroResp™
Technical Manual

MicroResp™ invented by Dr. Colin Campbell,
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A. Introduction

MicroResp™ is a unique microplate-based respiration system that enables the user to analyse up to 96 soil, sediment or water samples simultaneously and test a range of carbon sources and/or replicates in a small compact space. While sole C-source tests tend to select for fast-growing bacteria and rely on growth of organisms, MicroResp™ gives more immediate responses to these substrates and reflects activity rather than growth by measuring responses in the first 4–6 hours.

MicroResp™ reduces time, cost, and space demands in the laboratory, allowing users to test a broad range of experimental parameters in a rapid and reliable way. Although MicroResp™ was developed to measure CO_2 evolution over short periods (4–6 hours) it can be adapted for any time period depending on the activity of the sample.

This technical manual describes the general protocols for measuring CO_2 evolved from carbon amendments using an assay time of 6 hours and for soils <pH7. A range of materials can be analysed including volatiles, solid sources and ^{14}C sources. For the colormetric method, all the calculations and equation parameters have been determined using a Emax microplate reader (Molecular Devices, USA) and a 570nm filter. This methodology can be adapted to suit any experimental schedule, microplate reader and most filter wavelengths although re-calibration is required.

The optimum absorbance wavelength of the indicator is 572nm but due to instrument limitations 570nm is used and referred to throughout the manual.

B. Applications

MicroResp™ is currently being used to assess:

- Soil health and quality
- Toxicity testing
- Pesticide degradation profiles
- Community Level Physiological Profiles (CLPP)
- Pollution Induced Community Tolerance (PICT)
- Bioremediation evaluation
- Water ecology and toxicity

Other potential applications include:

- Microbe strain phenotyping
- Animal feedstuff digestibility
- Seed germination assays

Reference:

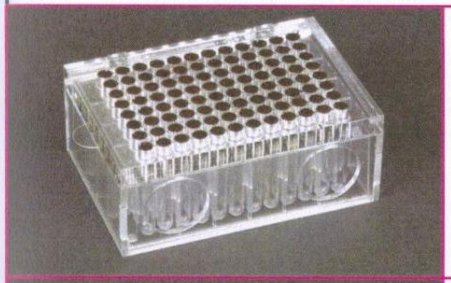
C.D. Campbell, S.J. Chapman, C.M. Cameron, M.S. Davidson and J.M. Potts. 2003
Applied and Environmental Microbiology, 69, 3593–3599.

C. Kit Components

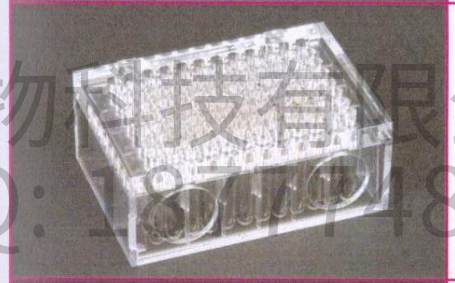
MicroResp™ Soil Respiration System consists of the following:

- 96-well 1.2ml Deepwell microplate
- 96-well microplate (Detection plate)
- MicroResp™ seal
- Filling device
- Metal clamp

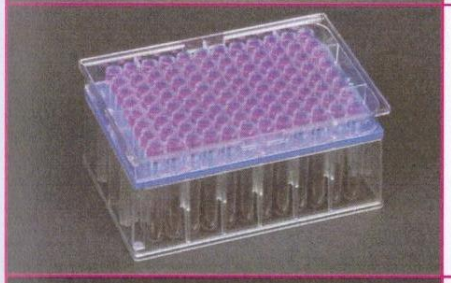
D. The MicroResp™ Procedure in Brief



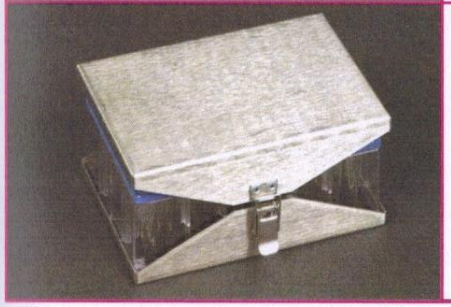
1. Place filling device over deepwell plate and fill with soil.



2. Remove sliding perspex sheet to allow soil to fall into deepwell plate.



3. Read detection plate at 570nm (0 hrs) and assemble onto the deepwell plate with the MicroResp™ seal.



4. Place in metal clamp and incubate at 25°C for 6 hours before re-reading detection plate at 570nm.

E. Equipment Required

General

- Soil conditioning unit (dark environment at a controlled temperature in which to incubate soil)
- 8-channel pipette(s) to dispense 25µl – 200µl aliquots (an automated pipette is recommended)
- 2mm stainless steel sieve

Colorimetric Method

- Spectrophotometer Microplate Reader (570nm filter)
- Desiccator (or other air-tight container)
- Hot-plate stirrer for detection gel preparation
- Water bath
- Microwave
- Balance (5 decimal place)

Radioactive Method

- Scintillation Counter (Microplate Reader)
- Syringe filter (0.2µm)

F. Safety Information

- Users should ensure that local safety regulations for all chemicals used are adhered to.
- Dispose of ^{14}C waste appropriately in accordance to your regulations.

G. Preparation of Soil Samples

Preparation

Sieve soils through a 2.0mm stainless steel sieve, removing roots and stones. A typical amount of soil required for a 96 well plate is 35–50g fresh weight. Store soil samples at 4°C when not in use. Determine soil moisture content[1] using a sub-sample of 5–10 g soil.

N.B.

1 Soils must not be too wet, as this restricts gaseous exchange, nor too dry, as this may adversely affect the microbial activity. For measuring microbial actvity, an acceptable range for the moisture content is 30–60% of it's maximum WHC[2]. Soils with an ideal moisture content should fall easily through the filling device.

2 If the soil moisture has been adjusted, incubate as described above for 5 days prior to the soils being used, and regularly check that the wick remains moist.

Incubation

Soil samples are incubated at 25°C for 3–5 days in a large sealed box containing a dish of self-indicating soda lime and lined with wet paper towels, prior to carrying out the MicroResp™ method.

There are two ways to carry this out depending on the preparation of the deepwell plates (see H4):

Method i. Fill the deepwell plates with soil samples as instructed and cover the plates with Parafilm®. During incubation check that the Parafilm® has not torn and replace if necessary.

or

Method ii. Place a wick (wetted paper towel) in the bag containing the soil sample and secure it above the sample using an elastic band so that half the wick is in the bag. Ensure that it does not come in contact with the soil.

N.B. *Method i. is advantageous for 6 + soil samples.*

H. Colorimetric Detection Protocol

H1. Preparation of Detection Plates

The agar and indicator solutions are prepared separately and are combined prior to use as instructed. The desired 1% concentration of Purified Agar is achieved when combined with the Indicator solution using 1:2 ratio (agar:indicator).

N.B. *150ml of agar/indicator makes up 7–8 plates. Always prepare a few extra plates.*

Method for Preparing Indicator Stock Solution

Dissolve the ingredients for the indicator solution in 900ml de-ionised (d.H_2O) over a low heat (≤65°C) before diluting to 1000 ml in a volumetric flask. Transfer to a bottle and store in the dark at 4°C for up to six months.

	Amount dissolved in de-ionised water	Final Detection Plate Concentration
Indicator Solution:	in 1000 ml:	
Cresol Red	18.75mg	12.5µg ml^{-1}
Potassium Chloride (KCl)	16.77g	150mM
Sodium Bicarbonate ($NaHCO_3$)	0.315g	2.5mM

N.B.

1 *The cresol red is yellow in solution and turns pink when the sodium bicarbonate is added*

2 *Do not heat or autoclave the indicator solution above 65°C*

Method for Preparing Detection Plates

1 Prepare 3% Purified Agar (3g per 100ml) in d. H_2O and dissolve by heating in a microwave on a low setting. Ensure the lid is loose during microwaving and gently mix at intervals. Check the volume has not changed and allow agar to cool in a water bath to 60°C.

2 Measure the required amount of indicator solution (2 times the amount of agar) into a bottle and warm in a water bath to 60°C.

3 Once the temperature of each solution has equilibrated, transfer the indicator solution and agar into a beaker and mix thoroughly, maintaining the heat at 60°C with constant stirring.

4 Dispense 150µl aliquots into six columns of the microplate using an 8-channel pipette (discard the first dispense back into the mixture). Repeat the procedure for the next 6 columns, and so on.

N.B. *Warm tips before use. When dispensing keep the pipette upright (not tilted). Place the tips in the wells so that when the agar mix is dispensed it rises up the end of the tips (immersing them in the agar mix). This reduces bubbles and also ensures the tip is "clean" when dispensing into the next well.*

5 Store the plates, in the dark at room temperature, in a desiccator with self-indicating soda lime on the base and a beaker of water. Leave uncovered for 1–2 days to allow to equilibrate, then cover each plate with Parafilm® if they are not used soon after.

6 Replace the soda lime when necessary and keep the atmosphere in the desiccator moist.

H2. Preparation of Substrates

The substrates are prepared as 30mg per gram of soil water.

To calculate the concentration of carbon source you need to know:

- weight (g) of soil in each well*
- moisture content of the soil

* *Record the weight of each soil weighed in the deepwell plate during filling or fill at least 6 wells of the filling device as described in Section H4.1&2 (but do not remove the tray), and divide the weight by the number of wells filled.*

Calculate according to the example below:

- If soil moisture content is 24.36% then there is 0.244g H_2O per g of soil
- If the weight of soil per well is 0.32g, then each well contains 0.078g H_2O
- If the substrate required is 30mg g^{-1} soil H_2O then the amount of substrate to be delivered in 25µl aliquots is: 0.078 x 30 = 2.34mg
- Dissolve 2.34g substrate in 25mls d.H_2O

If the variation of carbon source concentration between soils is small, an average concentration can be used (if desired).
The substrates are stored at 4˚C for up to 2 weeks.

List of commonly used substrates:

L-Alanine	D-(+)-Galactose	N-Acetyl glucosamine
L-(+)-Arabinose	D-(+)-Glucose	Oxalic acid
L-Arginine	γ-Amino butyric acid	Protocatechuic acid
Citric acid	α-Ketoglutaric acid	D-(+)-Trehalose
L-Cysteine-HCl	L-Lysine-HCl	
D-(-)-Fructose	L-Malic acid	

N.B. *Depending on the soil pH (i.e. less acidic) arginine may require to be used at 7.5mg g^{-1} soil water.*

H3. Template for Substrates

It is best to prepare a template of the deepwell plate demonstrating the positioning of the substrate replicates and soils. Note that the detection plate, which will be inverted on top of the deepwell plate, will read in reverse of the deepwell plate (i.e. position A1 corresponds to position A12). The deepwell plate is therefore set up in the reverse position of the desired display on the detection plate.

Deepwell plate filled as follows:

	Soil No 2						Soil No 1					
	1	2	3	4	5	6	7	8	9	10	11	12
A	cs9	cs9	cs9	cs1	cs1	cs1	cs9	cs9	cs9	cs1	cs1	cs1
B	cs10	cs10	cs10	cs2	cs2	cs2	cs10	cs10	cs10	cs2	cs2	cs2
C	cs11	cs11	cs11	cs3	cs3	cs3	cs11	cs11	cs11	cs3	cs3	cs3
D	cs12	cs12	cs12	cs4	cs4	cs4	cs12	cs12	cs12	cs4	cs4	cs4
E	cs13	cs13	cs13	cs5	cs5	cs5	cs13	cs13	cs13	cs5	cs5	cs5
F	cs14	cs14	cs14	cs6	cs6	cs6	cs14	cs14	cs14	cs6	cs6	cs6
G	cs15	cs15	cs15	cs7	cs7	cs7	cs15	cs15	cs15	cs7	cs7	cs7
H	cs16	cs16	cs16	cs8	cs8	cs8	cs16	cs16	cs16	cs8	cs8	cs8

Detection plate results correlate as follows:

	Soil No 1						Soil No 2					
	1	2	3	4	5	6	7	8	9	10	11	12
A	cs1	cs1	cs1	cs9	cs9	cs9	cs1	cs1	cs1	cs9	cs9	cs9
B	cs2	cs2	cs2	cs10	cs10	cs10	cs2	cs2	cs2	cs10	cs10	cs10
C	cs3	cs3	cs3	cs11	cs11	cs11	cs3	cs3	cs3	cs11	cs11	cs11
D	cs4	cs4	cs4	cs12	cs12	cs12	cs4	cs4	cs4	cs12	cs12	cs12
E	cs5	cs5	cs5	cs13	cs13	cs13	cs5	cs5	cs5	cs13	cs13	cs13
F	cs6	cs6	cs6	cs14	cs14	cs14	cs6	cs6	cs6	cs14	cs14	cs14
G	cs7	cs7	cs7	cs15	cs15	cs15	cs7	cs7	cs7	cs15	cs15	cs15
H	cs8	cs8	cs8	cs16	cs16	cs16	cs8	cs8	cs8	cs16	cs16	cs16

cs - carbon source

The above tables provide an example of how the experiment might be set up to measure the response of 2 soils to 16 carbon sources, in triplicate. It is recommended that samples are run at least in triplicate to ensure the best estimate of a mean absorbance (A_{570}) per carbon source. Include water to obtain the basal respiration.

H4. Preparing Deepwell plates

The deepwell plates can be prepared in two ways:
Method i. Soil samples are added and incubated in the deepwell plate(s) for 3–5 days prior to the addition of the carbon sources and detection plate
or
Method ii. Carbon sources are added to the deepwell plate(s) (as described in H5.3) prior to the addition of pre-incubated soil and detection plate

To fill the deepwell plate(s):

1 Insert the Perspex sheet into the filling device, and place the filling device on top of the deepwell plate.

2 Section off desired columns of the filling device with tape (if using more than one soil) before filling appropriate wells with soil. Sprinkle an excess of soil over the filling device and gently brush the soil into the wells until evenly filled, tapping the whole system once to gently compact the soil before adding more soil. Level off the soil and brush away excess soil.

N.B. *Do not force or press the soil into the filling device.*

3 Gently cover the section of soil filled, uncover the empty columns and fill with another soil as before. Once all the filling device is filled, remove all the tape.

4 Remove the Perspex sheet from between both plates, allowing the soil to fall through to the deep wells.

5 Place the Perspex sheet on top of the filling device and, using the fingerholds, gently but firmly tap the assembly on the bench so that any remaining soil falls into the deep-well plate. Any soil particles that have stuck will need to be pushed lightly down into the wells using a clean wire or rod.

6 Remove the filling device and for method (i) cover the deepwell plate with Parafilm® for incubation, or for method (ii) using pre-incubated soil, carry on to the next section immediately (miss H5.3).

7 To clean the filling device, wash by hand with detergent and rinse with deionised water, then dry.

H5. Assembling Components and Experimental Protocol

1 Switch on the spectrophotometer microplate reader.

2 Select your detection plates – check that the amount of agar in the wells of each detection plate is even and the colour consistent.

3 Allow the substrates to warm to room temperature. Use an 8-channel pipette to dispense 25µl of each desired substrate into the appropriate wells of the deepwell plate.

4 Apply the MicroResp™ seal to the deepwell plate(s).

5 Place the detection plate in the spectrophotometer and read the plate at absorbance wavelength 570nm.

6 Immediately place the detection plate onto the MicroResp™ seal by inverting the detection plate so that A1 corresponds to A12 on the deepwell plate. Apply firm, even pressure to seal correctly and secure the plates in a MicroResp™ clamp.

7 Save the time "At0" results to file and check the % coefficient of variance[3] (% CoV). Discard the plate if the % CoV is >5%, and read another plate.

8 Repeat steps 5–7.

9 Incubate the plates for 6 hours at 25°C.

10 After incubation, carefully disassemble the clamp, remove the detection plate and peel off the seal.

11 Immediately read the detection plate and save results "At6" to file as before.

12 The deepwell plate should be disposed of appropriately at the end of the experiment. The detection plates can be re-used as long as the agar has not dried and they have returned to their original colour and Absorbance reading.

13 MicroResp™ Seals are cleaned with detergent and rinsed with deionised water, then dried.

H6. Data Handling

i. Data Transfer and Sorting

Export the files from the spectrophotometer programme into an Excel spreadsheet and sort the absorbance (A_{570}) data into a list format with the 0hr (At0) and 6hr (At6) data in single columns alongside each other.

At0 plate

A1	A2	A3	...
B1	B2	B3	...
C1	C2	C3	...
...	...	...	...

At6 plate

A1	A2	A3	...
B1	B2	B3	...
C1	C2	C3	...
...	...	...	...

At0 and At6 in columns

At0	At6
A1	A1
B1	B1
C1	C1
...	...
A2	A2
B2	B2
C2	C2
...	...
A3	A3
B3	B3
C3	C3
...	...

ii. Normalisation of Absorbance data

Normalise the data (Ai) for time 6 by dividing the A_{570} data by the A_{570} data at time 0 (At0) and multiply by the mean of the A_{570} reading at time 0 (At0).

To Normalise:

6hr data: Ai = (At6 / At0) x Mean (At0)

iii. % CO_2 Calculations

The following formula converts the normalised 6hr data (Ai6) to %CO_2:

$$\%CO_2 = A + B / (1 + D \times Ai)$$

Where A = -0.2265, B = -1.606, D = -6.771

The formula is for a linear-to-linear (rectangular hyperbola) standard curve fit. The parameters are from a calibration using Emax Microplate reader (Molecular Devices, USA) using a wavelength of 570nm. The calibration was measured over 6h, using soils <pH7 and for pathlength (depth of agar) and concentration of reagents, in the detection plate as described.

N.B. *Calibration of the spectrophotometer is recommended as measurements differ from model to model (see Pg17).*

iv. CO_2 production rate

The CO_2 rate is calculated by converting the 6hr % CO_2 data to µg/g/h CO_2-C using gas constants, and constants for incubation temperature in °C (T), headspace volume (vol) in the well (µl), fresh weight (fwt) of soil per well (g), incubation time (hr) and soil sample % dry weight (dwt).

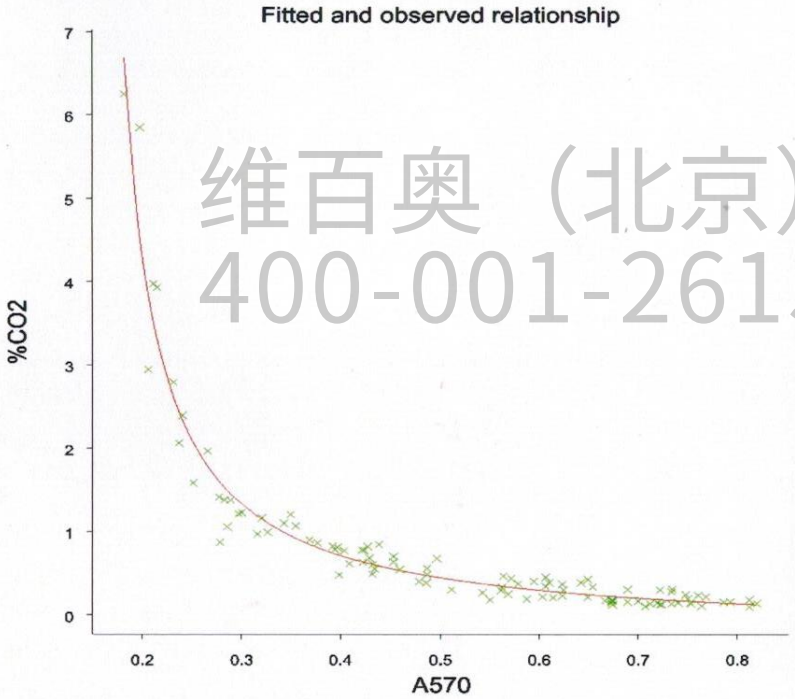
The CO_2 rate (µg CO_2-C/g/h) is calculated as follows:

$$\frac{\left(\frac{(\%CO_2/100) \times vol \times (44/22.4) \times (12/44) \times (273/(273+T)}{\text{soil fwt} \times (\text{soil \% dwt}/100)}\right)}{\text{incubation time}}$$

N.B. *Headspace volume is normally 945µl for our standard set-up. Adjust according to manufacturers specifications for the microplate and deepwell plate.*

v. Calibration of MicroResp™

The MicroResp™ system requires calibration for individual laboratories to take into account different spectrophotometers, different types of environmental samples, and incubation conditions. The conversion of Absorbance to $\%CO_2$ is a non-linear relationship, use a statistical package such as Minitab, Genstat, SPSS, etc. to carry out regression analysis (best fitting standard curve) to obtain the formula and parameters.



It is advisable to obtain a range of CO_2 and absorbance values to give the best possible results. There are various ways to carry out a calibration.

GC measurements

Duran® bottles (or similar) containing various volumes of soil (± carbon sources) and a Microstrip[4] of 4 wells (containing detection gel) are sealed with a rubber bung and incubated for 6h at 25°C. After incubation a sample of CO_2 is removed and measured on a GC. The bung is then removed and the strip of wells inserted into the carriage plate and read on the spectrophotometer.

Containers filled with different concentrations of $\%CO_2$ (see below) could also be used to calibrate the system.

Non-GC measurements

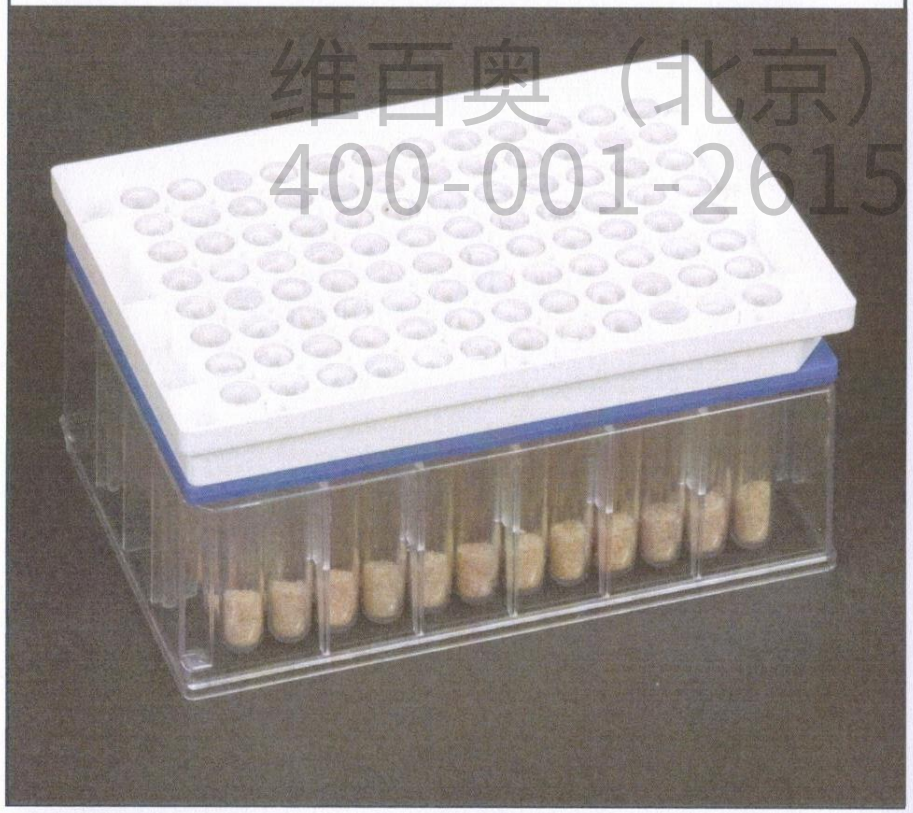
Prepare at least nine 40mL glass vials (Supelco 27180) with a Microstrip[4] of 4 wells and seal the vials. Remove from the vials the quantity of air equivalent to the quantity of CO_2 that will be inserted with a syringe for gas chromatography (see Table[5] Pg31). Fill a Tedlar bag with the standard, 5% CO_2 in Nitrogen (20DA gas cylinder, www.stgas.eu), and insert in each vial the corresponding quantity of CO_2. After 6 hours of incubation at 25°C, open the vials and read the Microstrip[4] wells on the spectrophotometer. The theoretical concentration of CO_2 and the absorbance reading from the spectrophotometer are used to obtain the calibration curve.

N.B. *The bigger the range the better the calibration.*

Alternatively, you could use IRGA or alkali trap or prepare mixtures in the laboratory by acidifying solid $CaCO_3$ in serum capped containers of known volume.

Reference: Rowel, M.J. Colorimetric method for CO_2 measurement in soils. 1995. *Soil Biology & Biochemistry,* 27, 373–375.

Radioactive Carbon MicroResp™



I. ^{14}C Detection Protocol

Please dispose of ^{14}C waste appropriately in accordance to your regulations.

I1. Preparation of ^{14}C-Substrates

1 Prepare the cold stock solutions[5] (i.e. non-radioactive) to 10ml and filter sterilise (0.2µm).

2 Add the required volume of radioactive C-substrate[6] from the stock solution to gain 6667Bq ml^{-1}. The target amount of ^{14}C per well is 200Bq, equating to a volume of 30µl. This equates to a total load of 19.2kBq per 96-well plate.

N.B. *As activity of the stock solution may start to degrade once opened, you may want to check the activity by measuring 3 x 30µl aliquots to determine the total amount added.*

List of commonly used ^{14}C substrates:

U-^{14}C-Arabinose
U-^{14}C-Arginine
U-^{14}C-Aspartic Acid
U-^{14}C-Benzoic Acid
1-^{14}C-Galactose
U-^{14}C-Glucosamine
U-^{14}C-Glucose
U-^{14}C-Glutamic Acid
U-^{14}C-Glycine
U-^{14}C-Lysine
1-^{14}C-Mannitol
N-acetyl-D-1-^{14}C -Glucosamine
Phenylethyl-1-^{14}C-amine
U-^{14}C-Sucrose
U-^{14}C-Xylose

Reference: Artz, R.R.E. *et al.* (2006)
Soil Biology & Biochemistry, 38, 2958 – 2962.

I2. Preparation of Detection Plates

To each well of an Isoplate-96 microplate add a rolled-up filter paper strip (Whatman No 1. cut to strips of approximately 15 x 5mm). It is advised to prepare the filter paper and the plates in advance.

N.B. *The filter paper sits at the edge of the well wall leaving the well bottom clear to allow for reading.*

I3. Preparation of the Deepwell plates

The deepwell plates can be prepared in one of two ways:
Method i. Soil samples are added and incubated in the deepwell plate(s) for 3–5 days prior to the addition of the ^{14}C substrates and detection plate
or
Method ii. ^{14}C substrates are added to the deepwell plate(s) prior to the addition of pre-incubated soil and detection plate

To fill the deepwell plate(s):

1 Insert the Perspex sheet into the filling device, and place the filling device on top of the deepwell plate.

2 Section off desired columns of the filling device with tape (if using more than one soil) before filling appropriate wells with soil. Sprinkle an excess of soil over the filling device and gently brush the soil into the wells until evenly filled, tapping the whole system once to gently compact the soil before adding more soil. Level off the soil and brush away excess soil.

N.B. *Do not force or press the soil into the filling device.*

3 Gently cover the section of soil filled and uncover the empty columns and fill with another soil as before. Once all the filling device is filled, remove all tape.

4 Remove the Perspex sheet from between both plates, allowing the soil to fall through to the deep wells.

5 Place the Perspex sheet on top of the filling device and, using the fingerholds, gently but firmly tap the assembly on the bench so that any remaining soil falls into the deep well plate. Any soil particles that have stuck will need to be pushed lightly down into the wells using a clean wire or rod.

6 Remove the filling device and for method (i) cover the deepwell plate with Parafilm® for incubation, or for method (ii) using pre-incubated soil, carry on to the next section immediately (miss I4.3).

7 To clean the filling device, wash by hand with detergent and rinse with deionised water, then dry.

I4. Assembling Components and Experimental Protocol

1 Add 40µl of 2 M NaOH solution to each well of the pre-prepared Isoplate-96 microplate.

2 Apply the MicroResp™ seal immediately to minimise evaporation.

3 Dispense 30µl of radioactive C-substrate into each well of the deepwell plate (refer to the template on page 10). Rinse the tip with the solutions once before dispensing.

4 Place the detection plate with MicroResp™ seal on top of the deepwell plate, firmly pushing down to seal. Ensure plates are positioned correctly so that A1 corresponds to A12 on the deepwell plate.

5 Secure the plates in the clamps and incubate the plates for 6 hours at 25°C. (This should be sufficient for normally active agricultural soils; excessively dry or wet soils may need longer incubation).

6 During incubation place the plates in a sealed box with soda lime to trap any leakage of $^{14}CO_2$. After use dispose of the soda lime via the radioactive waste route.

N.B. *Include water (Blank) to obtain the background count.*

I5. Reading Detection Plates after Incubation

1 Disassemble the clamp set-up.

2 Before counting the detection plate add 200µl per well of Optiphase 'SuperMix' scintillation fluid using a multi-channel pipette. The solution is viscous and requires two rinsing cycles before dispensing into the detection plate.

3 Seal the detection plate with a sheet of self adhesive microplate seal.

4 Read the detection plate as per instructions of the Scintillation counter plate-reader using counts per minute (cpm) and save to file.

N.B. *Total respired CO_2 normally does not normally exceed ~50% of the added C, hence a minimum of 9.6kBq remains in each deepwell plate after incubation. To ensure that no $^{14}CO_2$ escapes into the laboratory include approximately 3g of self-indicating soda lime in each waste bag of 8 soil-containing deepwell plates and check regularly that this is not exhausted.*

I6. Data Handling

Activity is determined from either counts per second or converted to Bq using a calibrated source in the same scintillation counter.

To calculate % C respired for each substrate:

$$\frac{\text{(final activity – background) x 100}}{\text{initial activity added}}$$

J. Frequently Asked Questions

Q. *Why do you use Purified Agar?*

A. Other agars tend to be cloudy (i.e. lots of particles, dead bacteria, etc.) and interfere with the Absorbance reading.

Q. *Why has my indicator solution and agar mix turned yellow during heating?*

A. You have overheated the mixture. It should not be heated above 65°C. Discard this solution and start again.

Q. *Why are my colorimetric detection plates yellow before beginning the experiment?*

A. The plates have been exposed to CO_2 during storage. They must be stored in a CO_2-free environment therefore replace the self-indicating soda lime frequently.

Q. *What are the limitations of the detection gel?*

A. Above 4–5% CO_2 the detection gel is becoming saturated (i.e. pale yellow) resulting in the $\%CO_2$ conversion being overestimated. If the detection gel is reaching saturation point for a particular carbon source reduce the concentration, or if the whole plate is reacting too quickly reduce the time period of measurement (i.e. 6h to 4h).

Q. *Is it possible to measure my samples for longer periods?*

A. Yes, although for longer than 24h you are in theory measuring growth of the micro-organisms rather than the initial substrate metabolic response. You also need to consider oxygen availability during longer incubation periods.

Q. *Can I use MicroResp™ to measure calcareous soils?*

A. Yes, with a modification to the method. The deepwell plates containing both soil and carbon source are exposed to the open air for a minimum of 30 minutes (or up to 2h) to allow abiotic CO_2 to be released prior to the addition of the seal and detection plate.

Q. *The wells at the edge of the plates have reacted differently to the wells in the centre.*

A. Most microplate methods have edge effects but we have found these to be fairly minimal. You can randomise your block design, but if you get very large variation which has nothing to do with the seal, it is likely to be temperature effects. You can purchase incubators that are specific for incubating microplates with very good air circulation, and have a large area available so plates are not stacked. It is good practice to check temperature control and variation in your incubator and as a compromise, try not to stack too many plates together (e.g < 3).

K. Product Use Limitations and Warranty

Unless otherwise indicated, this product is for research use only. Purchase of MicroResp™ products does not grant rights to reproduce, modify or repackage the products or any derivative thereof to third parties. James Hutton Limited makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery. Buyer's exclusive remedy and James Hutton Limited sole liability hereunder shall be limited to, at our option, product credits, refund of the purchase price of, or the replacement of all material(s) that does not meet our specification. By acceptance of the product, Buyer indemnifies and holds James Hutton Limited harmless against, and assumes all liability for the consequence of its use or misuse by the Buyer, its employees or others, including, but not limited to, the cost of handling. Said refunds or replacement is conditional on Buyer notifying James Hutton Limited within thirty (30) days of receipt of product. Failure to give said notice within thirty (30) days shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s).

L General Information

Technical Support and Ordering Information:
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Mailing Address:
James Hutton Ltd
Craigiebuckler Aberdeen AB15 8QH UK

Supplied by us:

Component	Catalogue No.
Starter Kit (includes 1x MicroResp™ Seal, 1 x Filling Device with sliding tray, 1 x Metal clamp, 5 x 96-well 1.2 ml Deepwell plates*, 5 x 96-well Microplate*, 1 x 96-well MicroStrip plate*, 1x Technical Manual)	001
MicroResp™ Seal	002
Filling Device with sliding tray	003
Metal clamp	004
Technical Manual	005

*These are supplied only as part of the starter pack.

External Suppliers:

Consumables	Supplier	Product Code
Deepwell plate 1.2 ml (96-well round bottom) pk50	Fisher	12114172
Microplate (96-well flat bottom) pk50	Fisher	12607755
Breakable Microplates, pk50	Fisher	10557915
For 14C MicroResp:		
Optiphase 'SuperMix' scintillation fluid, 5L	Perkin Elmer	1200-439
Plate seal for microplates, pk100	Perkin Elmer	1450-461
Isoplate 96 (^{14}C only), pk50	Perkin Elmer	6005040

M. Acknowledgements

The Macaulay MicroResp™ team held a workshop on 26–28 September 2005 to discuss *"Development of methods for assessing belowground biodiversity as an indicator of soil quality and health"*. We would like to acknowledge the positive feedback and constructive comments from all the participants which have helped to improve the manual and methods contained in it. In particular we would like to acknowledge Nahia Gartzia Bengoetxea (NEIKER-Basque Institute) for highlighting the large improvement in using a 570nm filter as opposed to 590nm.

Notes

1. Soil Moisture Content and dry weight (dwt)

5–10g soil is oven dried at 105°C for 24 hours.

To calculate % dwt:

$$\frac{\text{Dry weight of soil}}{\text{Fresh weight of soil}} \times 100$$

To calculate % soil moisture content:

$$100 - \%\ \text{dwt}$$

2. Water Holding Capacity (WHC)

The maximum water holding capacity of a soil is the amount of water the soil can hold when saturated. Use an appropriate soil handling protocol to adjust the moisture content of the soil.

3. % Coefficient of Variance (% CoV)

To calculate % CoV:

$$\frac{\text{standard deviation}}{\text{mean}} \times 100$$

4. Microplate strips (MicroStrip)

Assembled 1 x 8 well breakable strips in a microplate frame are filled with the detection gel. These can be broken (usually into a strip of 4 wells) and placed into a sealed jar or suitable container for the measurement of CO_2.

Notes

5. Non GC Calibration Table

Example range of CO_2 concentrations for a calibration curve

Final CO_2 conc. (%)	Remove air (mL)	Insert 5% CO2 (mL)
Ambient (~0.039)	0	0
0.0625	0.5	0.5
0.125	1	1
0.25	2.5	2.5
0.625	5	5
1.25	10	10
2.5	20	20
3.75	30	30
5	40	40

Notes

6. Calculation of Non-radioactive "cold" stock solution

Non-radioactive ("cold") stock solution is added to soil so that the substrate consumes between 100 and 70µl of O_2 per well assuming complete oxidation over 144h of incubation (Campbell *et al.*, 2003) and the wells do not become anaerobic during the incubation.

To calculate the concentration of cold stock solution, follow the example below.

Example: Glucose $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$

From the equation 6 moles of O_2 per mole of glucose is required for complete oxidation
If the volume of O_2 in each well = 0.070ml and the molecular weight of glucose = 180.16g
Therefore,
mg substrate per well
= (vol. O_2 $well^{-1}$ x Mwt substrate)/(22.4L mol^{-1} x moles O_2)
= (0.070 x 180.16)/(22.4 x 6)
= 0.0938mg $well^{-1}$

If the volume substrate added to each well is 30µL, the cold stock concentration is:

$\frac{0.0938}{30} \times 1000 = 3.1g\ L^{-1}$

= 0.031g in 10mls

Notes

7. Calculation of ^{14}C substrate

Radioactive stock is mixed with cold substrate to give 200Bq per well.

Example:
If the radioactive stock is at 1.5MBq in 1ml
the volume of cold stock is 10ml,
and volume added per well is 30µl

The total activity required in 10ml
= 200Bq x 10000µl/30µl = 66666.7Bq

Therefore, the amount of radioactive stock to add to the cold stock solution:

$$\frac{66666.7\text{Bq} \times 1000\mu\text{l}}{1.5 \times 10^{6}\text{Bq}} = 44.4\mu\text{l}$$