



SOP 2019
DCR Models 701.2 +

SOIL CO₂ RESPIRATION OFFICIAL SOLVITA® INSTRUCTIONS

TESTING SOIL RESPIRATION: The release of carbon from soil as CO₂ due to the action of soil organisms is a dynamic feature of all soils and is particularly noticeable in soils managed biologically. This activity reflects the interplay of microbes with *added and sequestered* carbon. CO₂ respiration therefore has broad applications in agriculture and ecology.

Solvita® is a protocol developed to enable any agricultural lab to measure short-term soil respiration and complements the two other Solvita tests (SLAN and VAST) enabling soil health assessment. Two ways of evaluating CO₂ include: **CO₂-Burst**, the chosen application for soils previously dried and sieved as is common for soil labs, and **Basal**, which measures CO₂ respiration in fresh, minimally disturbed soils. **CO₂-Burst** is most suited for processed, dry and stored soils, and is appropriate ecologically for regions with intermittent dry-wet soil cycles. **Basal** requires more careful sampling* and reflects natural, background microbial rate, and therefore may more accurately describe soils especially in moist, temperate climates. It is advisable to first identify the intended use of the information then select the most suited Solvita method.

IMPORTANT METHOD UPDATES IN THIS 2019 MANUAL:

1. **REDUCED SOIL VOLUME for DYNAMIC RANGE ADJUSTMENT**

Effective immediately, Solvita 2019 utilizes a standard 30 cc soil scoop (included with new DCR purchase and also available separately). The 40g weight method is no longer supported. This change reduces the potential for high CO₂ results during CO₂-Burst tests and significantly improves throughput capacity without a loss in quality.

2. **INCUBATION HEADSPACE ADJUSTMENT**

Solvita 2019 now employs and recommends a standard jar size of 475 cc (included with new purchases and available separately). The combination of these two changes has been shown to significantly improve range and reliability of Solvita tests.

SOIL HEALTH LAB LISTING PROGRAM

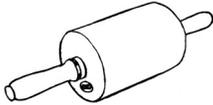
Solvita encourages all persons and labs undertaking these tests to become officially listed in the Solvita Soil Health Proficiency Program. This involves a voluntary QAQC review annually with reference soils provided by Woods End Labs. See the section at the end of the manual to apply.

* A separate field test SOP manual is available from Solvita.com/fieldtest/

2019 PROTOCOL (SOP)

1. **SOIL SAMPLING:** Use your preferred strategy for soil sampling and sampling-depth selection. To best represent any area take a minimum of 12 individual cores for blending into a single sample. Dried, stored soil may also be used. For basal sampling see separate manual.

A soil roller is recommended to crush soils



A standard soil scoop (30 cc) is employed to measure soils



2. **DRYING:** To obtain a CO₂ flush the soil must be dry (less than approximately 3% moisture). Otherwise the burst response will be compromised. Temperatures up to 45°C are acceptable.

3. **SAMPLE PREPARATION:** All soils should be minimally processed to avoid artifacts especially caused by grinding & machine crushing which influences the biology. Soil rollers (available from Solvita) are optimal to crush and break soil clumps if needed. For most sandy or silty soils simple sieving may be all that is needed. Sieve soils between 2 and 6 mm.

QC recommendation: Practice consistent technique by evaluating results using different soil processing methods in your lab.

4. **MEASURE STANDARD VOLUME OF SOIL:** In **CO₂-Burst** mode, measure soil with 30 cc volume scoop. When scooping dry soil from a larger container, premix the soil. Overfill the scoop slightly, and then strike the soil level with the striker included with scoop. Pour this soil into the plastic beaker and gently tap to allow soil to become level, before addition of water.



Pour dry soil into internal beaker.

5. **ABOUT ADDING WATER:** Dribble 9 cc water onto each 30 cc sample inside the beaker. This is a modified water-filled pore space (WFPS) method. 50% of WFPS simply means filling half the available soil volume with water enabling microbes sufficient air space.



Place beaker containing 30cc soil into the glass jar before moistening.

QC recommendation: The chart at left shows that by using 9 cc water for 30 cc volumes having varying bulk-densities the actual WFPS obtained will vary between 44 and 54% which is within the optimal biological range. You may practice adding differing amounts.

(A)	(B)	(C)
30 cc Soil weight grams	Water, to fill WFPS cc	50 % of WFPS
25	9	44%
27	9	46%
29	9	47%
31	9	49%
33	9	52%
35	9	54%

CTIONS



Add DI water to soil by slow dribbling to reach 50% of available pore volume or 9 cc for 30 cc soil



Insert CO₂ probe

Probe color change may be viewed from outside the jar during incubation.



At 24 hrs read the probe result by inserting into the DCR

6. **WATER PIPETTING:** For rewetting dry soils, dribble water onto the soil surface (picture) using care not to form a crater by delivering water too rapidly or by muddying the soil surface.

QC recommendation: Practice adding water to 30 cc samples of differing soils and allow to settle to observe the distribution of water throughout the soil sample. Cautionary: Outlier soils are likely to contain very high organic matter or high clay content.

WATER DISPERSION NOTE: To help dispersion of water when adding from the top to soil a plastic screen is provided in the Solvita kit which is placed on top of the soil while dispensing. Once the water is added it should equilibrate rapidly downwards.

7. **STARTING TEST:** Open the Low-CO₂ probe pouch by tearing the foil open and carefully remove the plastic probe without touching the gel. Place the probe into or alongside the soil with the gel facing out (pictures). If doing a basal test, simply insert the probe directly into the moist soil. *Tightly screw on* the lid which contains a red gas-tight gasket. Record the start time. Keep the jar under stable room temperature or incubator set at 20°C.

QC recommendation: Soil respiration depends on temperature. If the test is run higher than 20°C this may be unrealistic in relation to the actual average daily soil temperature in the entire topsoil layer.

8. **READ THE COLOR RESULT:** At 24 hrs open the jar, remove the detector probe and read the color number by inserting the probe face-up into the Solvita Digital Color Reader (picture). For the **CO₂-Burst** application choose the **CO₂-Low button**. For a **Basal** test, the ALT key is used (consult the Field Respiration Manual).

9. **DIGITAL COLOR RESULT.** The DCR reports two values: the Solvita color number based on measured optical density of the test gel and then interpolated to CO₂-C ppm based on the *recommended test parameters*, see also Table 2.

QC recommendation: You may develop your own Solvita method by calibrating the color output number (optical density) to any other condition of soil or jars by keeping in a range of 0 - 3% total CO₂.

> **SOLVITA 2019 ACCEPTABLE JARS:** The new Solvita jar with CO₂ gasket is 475 mL (1-pint). Other acceptable jars include Ball 1-PT canning jars and lid (these lids must be replaced frequently as the thin rubber seal affixed to the lid becomes damaged). Also accepted are 475 mL Schott jars (standard Solvita IRTJ jars).

TABLE 2. RANGE OF RESPONSE GUIDELINE SOP 2019

SOLVITA TEST COLOR	CO ₂ ppmv in jar head-space	Soil Result CO ₂ -C as mg/ liter	Soil Biological Fertility Classes using standard Solvita 2019 with Volume Scoop and 475 mL
5	30,000	200	Typical High Biology Soil, Strong N-min Potential
4	10,000	75	Medium Biology Soil, Some N-min Potential
3	3,000	30	Medium-Low, Low N-min Potential
2	1,000	15	Typical Low Biology Soil, Very Low N-min Potential
1	500	< 5	Soil Very Low in Microbes, No N-min Potential

Solvita® Color Response

Solvita is a colorimetric gel chemistry carefully designed to indicate the concentration of carbon dioxide accurately over a range of 0 - 3% as CO₂ (0 - 30,000 ppmv) and is particularly sensitive in the 0.05% to 0.5% range. When plotted on a log scale the Solvita color should yield a perfectly linear result (see Fig. 1 based on 51 soils from NC, VA, OK in 2018). To obtain the final soil result as mg/kg or mg/liter Solvita employs the Ideal Gas Law @ STP 20°C, 1 ATM.

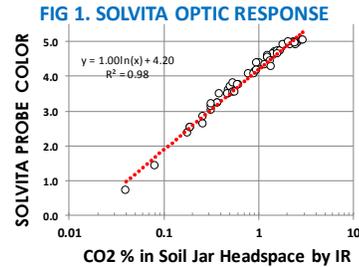
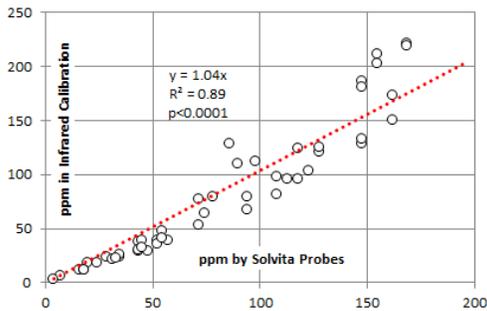


Fig 2. Soil CO₂-C: IRGA vs SOLVITA in 475 cc JARS



Studies conducted with this new Solvita-2019 SOP show that users may expect to obtain virtually identical results for soil CO₂ by Solvita compared to methods such as static infrared (IRGA) detection (see Fig. 2).

INTERPRETATION: The useful range of Solvita CO₂ response depends on the use. However, based on decades of experience in soil respirometry for agricultural soils the range of expected results should generally fall between 5 to 150 mg/kg/day as CO₂-Burst (and 2-4x lower as **Basal** rate).

Soils that lie outside this normal range (forest soils, histosols and intensive organic) may need an adjusted method (change in soil volume and change in jar constraints). Woods End Labs has also determined that it is very difficult to obtain reliable respiration results by miniaturizing sample size for respiration lower than 10 g per test. Solvita technical staff can be helpful for persons wishing to calibrate Solvita to other conditions.

Solvita is not responsible for results gained by non-approved methods or by any SOP earlier than the current.

CARBON and N-MINERALIZATION vs Methods

CO₂-Burst as used in this protocol evaluates biological responsiveness by simulating a drying-rewetting event, *analogous to rainfall after a very dry or droughty period*. Such events do not necessarily occur in all soil zones, if at all. **Basal** respiration represents the biological microbial rate typical of a more steady-state system found in a moist, temperate climates. Calculations of the carbon turnover or N-mineralization based on extrapolating these differing test results must take into account the ecological significance of the protocol. While **CO₂-Burst** is believed to be a natural phenomenon documented by many soil ecologists and varies during the course of the year, it is also strongly influenced by soil disturbance particularly lab grinding and sieving.

Basal respiration possesses different limitations, chiefly those affected by the quantity of moisture that happens to be present in the soil at the time of testing. Despite this, basal is likely a more realistic ecological index of soil microbial activity over time and is potentially more useful for extrapolation.

Applying any one of these tests to predict C-min or N-min requires several assumptions including how many pulses occur per season (if **CO₂-Burst**), or how long soil is warm and moist (**Basal**). Solvita provides an on-line calculator (solvita.com/soil/basal-co2-guide/) which enables one to input average soil temperature and the number of days soil is either too wet or too dry which influences the calculation of potential C-min and N-min. Additionally, soils need to be evaluated for organic-N by the Solvita SLAN test which indicates potentially labile nitrogen. Soils low in SLAN may be innately poor at releasing nitrogen. The efficiency of N release per unit of carbon also depends on the C:N of the soil-crop system (a factor in the on-line calculator) as well as microbial diversity, since species have differing metabolic N-use requirements. Therefore, any respiration test is an approximation of the annual behavior.

Solvita[®] Reference/Proficiency Program and DCR QAQC

Labs implementing respiration testing can monitor Solvita performance by participation in the Solvita Reference Program (contact: solvita@woodsend.com). Soils of known management history are provided which vary in soil health. Participants provide Solvita results of replicate tests and note variables during the test. QAQC is now also available for DCR performance separately using *Optic Check Cards*. Desired precision is within ± 1 MAD and outlier boundary ± 1 SD. Labs fulfilling criteria are eligible for *Solvita Certification* and lab recognition.

Solvita DCR's are identified by a serial unit number and a firmware revision level. This rev should be noted when reporting results and DCR's should be kept current with updates that are offered free by the company. DCR's may be attached by USB cable to a PC for uploading test results.

TABLE 3. TROUBLESHOOTING GUIDELINE

Indicated Issue	Possible Explanation	Possible Remedy
Solvita Color is high	Soil is very high in organic matter or with high respiration quantity	Consult Solvita staff. Use less soil or larger volume and adjust results
	Old 40 g method being used	Switch to new 30 cc method
Solvita color is low	Soil is very depleted	Allow soil to go longer in test
	Old capillary-wetting method used	Use improved WFPS method
	Jar gasket missing or leaking CO ₂	check seal and re-run test
Solvita test indicates high respiration but plant growth is not satisfactory	Nutrient imbalances are involved not related to soil health	Test for pH, N, P, K and possibly add these nutrients if indicated
	C:N ratio of newly added organic matter is high and ties up N	Wait to test after steady-state conditions return
Solvita test indicates low activity even though organic matter was added recently	Soil pH may be low and is depressing microbial response	Check acidity and correct with limestone if indicated
	Added organic matter is not labile and is not metabolized by microbes	Retest later after organic matter has had time to acclimate to soil factors
Solvita test result declined after the soil was fertilized	Fertilizer salts may be excessive or are limiting soil microbes	Check salts in soil and leach with water if excessive
	Ammonium fertilizer caused drop in soil pH which lowered activity	May require limestone to compensate if condition persists
Solvita test low at first but is high shortly after adding organic matter	A very high application of active organic matter may have occurred	Use organic matter more sparingly or use more stable manure or compost
	A clump of organic matter/manure may have been put in test jar	Re-mix soil sample and be careful to avoid clumps
Solvita test is variable with differing samples from same area	Soil is strongly profiled, non-homogenous or contains clumps of sod or other organic matter	Re-design sampling plan; take more grab samples, watch depth of sampling and sieve more carefully
Solvita test was very low after water added	Excessive water was added and microbes are limited for air	Use water-filled pore space method or calculate water capacity carefully
For CO ₂ -Burst test, soil did not wet properly	Soil is moderately hydrophobic or high in clay content	Allow more time for remoistening before starting the test

Early Model DCR's: It is necessary to obtain firmware upgrades for early models to meet current rev level 701.2. Models prior to this must be returned for recalibration. Models earlier than 700.6 (series 600, 500 etc) require hardware revisions and recalibration. Pouch Longevity: Solvita probes are packaged to assure freshness for up to 1 year at room-temperature 20C storage. High heat (>30°C) reduces the storage life. For storage longer than 4 months, use cool (5-10°C) conditions. Do not allow to freeze which voids the warranty.